STUDIES IN THE SEPARATION OF SMALL AMOUNTS OF INORGANIC SUBSTANCES USING CHROMATOGRAPHIC PROCESSES

By

James B. Headridge, B.Sc., A.R.I.C.

Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

July, 1956. University of Edinburgh.

TABLE OF CONTENTS

Page

INTRODUCTION

Due to the ever-increasing use of organic complexing reagents in chemical analysis, it is now possible to obtain a quantitative separation of many elements in a short period of time. Much of the time-consuming work, which had to be done, if such separations were to be accomplished at the beginning of the century, is no longer necessary. However, there are few instances of complex formation occurring with the Group IA elements and, although complex formation with the Group IIA elements is widely found, the complexing reagents have shown a lack of selectivity towards these elements. The methods of complex formation have therefore produced the best results, when applied to the metals that are in periodic groups other than IA and IIA, and it is still generally recognised that, with the exception of the rare earths and the pairs, zirconium-hafnium and niobium-tantalum, the elements in the groups, potassium-rubidium-caesium and calcium-strontium-barium are the most difficult to separate.

A complete quantitative separation of the metals in these groups by classical methods is still a lengthy procedure. The following passages are taken from the textbook on analysis by Hillebrand and co-authors[1], who mention the

difficulties that arise in a separation of these elements.

" . . . . . there appears to be no truly special reagent for either rubidium or caesium ions, whereby these can be quantitatively separated from potassium or from each other. Differences in the solubilities of certain of their salts serve as a means for obtaining each of them pure, but the methods are in no sense quantitative."

"The determination of any one of the alkaline earth metals, when free from the others, is relatively simple, but, when two or all three are associated, it is otherwise, for their separation is then influenced by their relative proportions. No really good methods for separating calcium, strontium and barium from each other are known. They are all imperfect and give correct results only through compensating errors."

The aim of this work was to examine the possibilities of finding speedier methods for the determination of these elements, by making use of chromatographic processes.

The greater part of this thesis is therefore taken up with studies on Groups IA and IIA of the Periodic Table. The remaining part deals

with certain other elements, in particular, those of Group IIIB.

Three mechanisms can be distinguished under the general heading of chromatography, namely, adsorption, partition and ion exchange. A separation process is seldom governed by only one mechanism, but with most procedures, one of these mechanisms predominates and gives its name to the separation technique. In the following paragraphs, the use of the three methods is discussed with regard to the elements in Groups IA and IIA.

Adsorption: This mechanism has seldom yielded satisfactory separations, but, recently, the separation of microgram quantities of strontium and barium in a column of specially activated alumina has been achieved[2]. The barium alone was retained from a neutral solution of the alkaline earths when the column was washed with water.

Partition: This involves paper chromatography and cellulose column work. Paper chromatography was first introduced as a separation method by Consden, Gordon and Martin in 1944[3]. They used it to separate mixtures of amino-acids. It has been applied in the field of inorganic chemistry, since 1948. There are few references

to the separation of inorganic cations of Groups IA and IIA on cellulose columns, but there is little doubt that separations achieved on paper strips could be carried out on a large scale, on such columns.

The separation of lithium, sodium and potassium is by no means difficult and references to this separation are numerous. Chlorides and nitrates have been most widely used and are generally separated with solvents consisting of one or more alcohols, e.g. methanol- n-butanol (80 : 20% V/V)[4]. To separate potassium, rubidium and caesium is a much more difficult task and separations of these elements are mentioned only twice in the literature[4], [5]. Concentrated hydrochloric acid - methanol - n-butanol - methyl isobutyl ketone (55 : 35 : 5 : 5% V/V)[4] and the phenol-rich layer from liquid phenol - 2N hydrochloric acid (1 vol. : 2 vol.)[5] have been used as solvent mixtures.

Despite the many trials with solvent-mixtures to separate beryllium, magnesium and the alkaline earths, few have been successful[4], [6], [7], [8], [9], [10] and it is doubtful if any of these are entirely satisfactory. Pollard and co-workers[7] used symm. collidine (2 : 4 : 6

trimethyl pyridine) - 0.4N nitric acid (50 : 50% V/V) to separate magnesium, calcium, strontium and barium, and Miller and Magee[4], concentrated hydrochloric acid - n-butanol (95 : 5% V/V) for the separation and semi-quantitative estimation of strontium in the presence of magnesium and other alkaline earths. Erlenmeyer and co-workers[8] reported the separation and determination of alkali and alkaline earth ions with ethanol - 2N acetic acid (80 : 20% V/V) but the R_F values given, show that a complete separation of either group was not achieved. The use of tertiary butanol - 6N hydrochloric acid (50 : 50% V/V) has been advocated by Surak and co-workers[10].

Ion Exchange: The alkali metals have been separated on ion exchange resins[11], [12], [13], [14], [15]. In most cases, the mixtures were taken on to a column of a cation exchange resin in the hydrogen form and the column eluted with dilute hydrochloric acid. However, lithium, sodium and potassium have been separated on a column of Amberlite IR - 120 with uramil diacetic acid as eluant[13], differences in the stabilities of the complexes formed with these ions, yielding a separation.

No complete separation of the Group IIA elements on ion exchange resins has been reported up to December 1953 although some work has been done on the separation of strontium, barium and radium in the United States in connection with the Manhattan Project[16], [17]. Tompkins[17] was able to separate 20 mg. strontium, 20 mg. barium and 20 µg. radium on a column of colloidal Dowex 50 by eluting with ammonium citrate solution.

In chromatographic elution, the salts may be detected in the effluent by measuring change in conductivity or activity, if radioactive isotopes have been employed, or determining the amount of constituent in small volumes of the effluent by evaporation to dryness. The first two methods require complicated physical apparatus and the last method can only be used in a restricted number of cases. It therefore appeared, from a study of the papers on the separation of the elements of Groups IA and IIA on ion exchange resins, that the most satisfactory method of separation would be to strip one cation from the column while not appreciably moving the others.

Initially, therefore, investigations were carried out on the possibilities of achieving a

separation of the alkali and alkaline earth elements using the ion-exchange chromatographic method. A study of the complexing properties of various reagents indicated that 8-hydroxy-quinoline might be a suitable eluting substance.

Failing to achieve a speedy and effective separation of these elements employing ion exchange resins, it was the intention to examine fully, the field of partition chromatography, the feeling being, that in these two branches of the chromatographic process, indicated above, there were possibilities of better separations. Improvements in existing procedures would require a detailed investigation of solvent-mixtures and conditions of operation.

By whatever method the separation was achieved, it was decided that the procedure evolved, must be capable of allowing the detection and semi-quantitative estimation of 0.1 μM (micro-moles) of a particular cation in the presence of 10 μM of the others, singly or combined, and if possible be suitable of application to the quantitative determination of the constituents in natural materials.

ASSEMBLY FOR THE COUNTING APPARATUS.

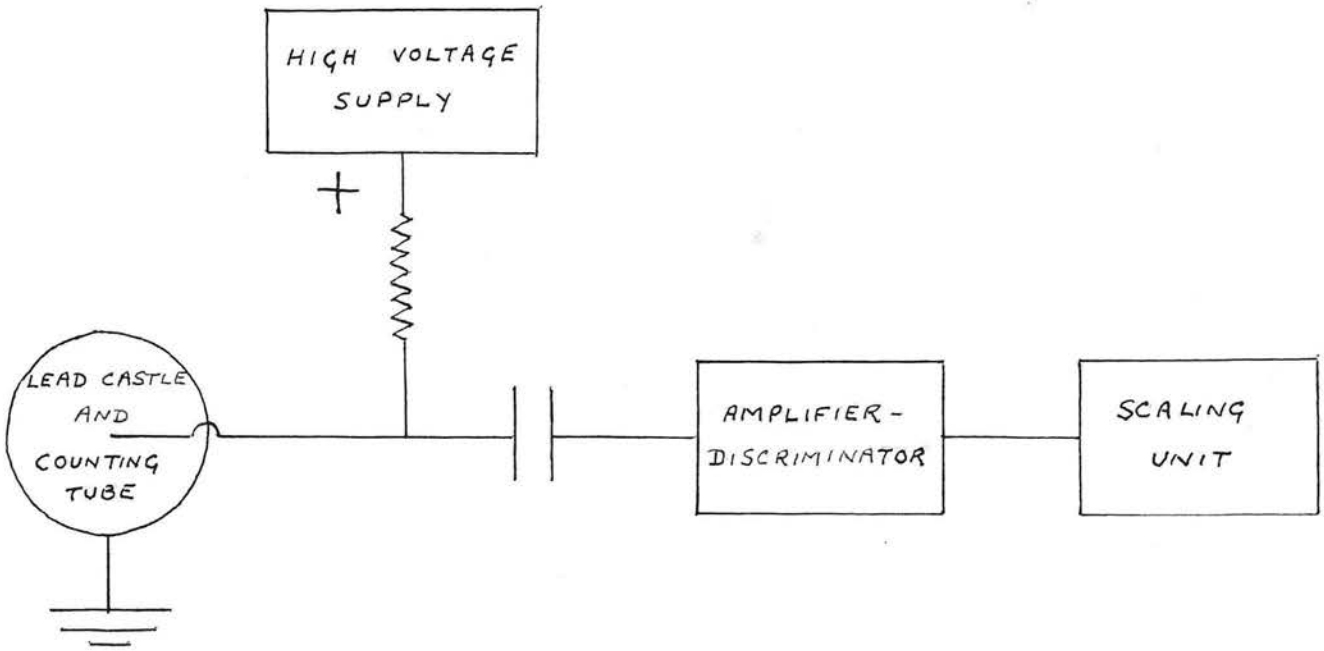


FIGURE 1.

EXPERIMENTAL

APPARATUS

Radioactivity Counting Apparatus: The activities of the radioactive solutions were measured with the apparatus whose components are shown in Figure 1. This apparatus was constructed in the Chemistry Department, Edinburgh University. It was very effective for β radiation and, in general, an M6 liquid counter (185 mm. x 23 mm., capacity 9 ml., supplied by Twentieth Century Electronics, Ltd.) was used.

Ion Exchange Resin Column: For work with ion exchange resins, a Pyrex glass tube (length 45 cm., internal diameter 1 cm.) was constructed with a stop-cock at its lower end. The resin column was supported and retained by plugs of glass wool.

Chromatographic Vessels: Apparatus fitted for descending chromatography was used. Much preliminary work was carried out in a cylindrical jar, 44 cm. long and 10 cm. in diameter. This stood on a ground glass base plate and contained a stand supporting a glass boat, $6\frac{1}{2}$ cm. in length with a capacity of 15 ml. In this apparatus, a run of 40 cm. could be achieved. After the correct solvent-mixtures were established, chromatograms were run in a 9"

Shandon Chromatank, having an overall height of 22" and a solvent trough 7¾" long. This vessel accommodates sheets 16 cm. wide with a run of 50 cm.

Oven: A thermostatically-controlled, electrically-heated oven was used to dry the strips before they were sprayed. The strips were suspended in such a way that no part of them came in contact with the inside surface of the oven.

Strip-holder: For spraying purposes, the strips of filter paper were stretched taut in air between two blocks of soft wood covered with absorbent paper. Drawing pins were used to attach the strips to the blocks.

Spray: A simple atomiser actuated by a current of compressed air was used.

Chromatographic Papers: Sheets or strips of Whatman Filter Papers Nos. 1, 3MM, 4 and 41 were used.

Apparatus for applying Solutions to Paper Chromatograms:

(a) Glass Capillary tubes and 'Agla' micrometer syringe - For qualitative work, the test solutions were applied to the paper from glass capillary tubes calibrated by means of an 'Agla' micrometer syringe (supplied by Burroughs

Wellcome and Co.). For quantitative work, the solutions were applied to the paper from the syringe fitted with a glass capillary nozzle, provided that the volume of test solution was 0.3 ml. or more.

(b) Micro-pipette with 'Pumpett' - For quantitative work, if 0.3 ml. of solution was not available, the solution was applied from a micro-pipette used in conjunction with a 'Pumpett' automatic pipette control (supplied by the Shandon Scientific Company).

Glassware: As far as possible Pyrex ware was used. The stock solutions of the salts of the elements under investigation were contained in glass-stoppered Pyrex bottles.

Porcelain ware: Evaporations to dryness were usually carried out in 2½" Royal Worcester porcelain basins.

Platinum ware: A 50 ml. platinum basin, a 6 ml. platinum crucible with its supporting platinum triangle, and platinum-tipped forceps were used when necessary.

Sieves: For sieving ion exchange resins, a set of micro-sieves (supplied by Gallenkamp and Co. Ltd.) were used.

Ultra-Violet Lamp: A 'Hanovia' lamp (300 watts) was used.

Balance: A Stanton Model A.D.2 analytical balance, sensitive to 0.1 mg. was used for all weighings.

pH Meter: All pH measurements were made on a Cambridge pH meter.

Spectrophotometer: A Unicam SP 500 Spectrophotometer was used for various purposes. The accessories that were used with this instrument for spectrophotometric titrations are described in the text.

Apparatus for maintaining constant room temperature: By means of a steam radiator, supplemented as required by electrical tubular heating controlled by a bimetallic strip thermostat and hot-wire relay switch, the temperature of the laboratory was maintained at about 18°C while chromatograms were being run.

CHEMICALS

Radioactive Salts and Solutions:

Rubidium 86 chloride (half life 19.5d.; a β, γ emitter; specific activity 1.9 mc/gm.) and caesium 134 carbonate (half life 2.3y.; a β, γ emitter; specific activity 1.6 mc/gm.) were obtained from the Atomic Energy Research Establishment at Harwell. Solutions of the chlorides were prepared with activities of about 10,000 counts / min. /ml.

Standard Solutions: Molar solutions of the Group IA and IIA cations were prepared from the following materials.

Lithium - Merck's "Extra Pure" lithium carbonate in hydrochloric acid.

Sodium - "Analar" sodium chloride.

Potassium - "Analar" potassium chloride.

*Rubidium - "Specpure" rubidium chloride.

*Caesium - "Specpure" caesium chloride.

Beryllium - Kahlbaum's beryllium oxide in hydrochloric acid.

Magnesium - Hilger "High Purity" magnesium metal in hydrochloric acid.

Calcium - "Analar" calcium carbonate in hydrochloric acid.

Strontium - "Specpure" strontium carbonate in hydrochloric acid.

Barium - "Specpure" barium chloride.

* At first, molar solutions of rubidium and caesium chlorides were prepared from ordinary grade rubidium and caesium carbonates. It was later found, from paper chromatograms, that these rubidium and caesium solutions contained traces of caesium and sodium respectively. From then on, solutions of "Specpure" chlorides were used.

1% W/V solutions of rubidium and caesium ions were available for researches on complex formation with 8-hydroxyquinoline. These had been prepared from "Specpure" rubidium and caesium chlorides.

Other Test Solutions: 1% W/V solutions of the other cations were prepared from Salts of Analytical reagent grade.

Reagents: The acids, alkalis and other inorganic reagents used were, as far as possible, of "Analar" standard. The organic solvents were purified and fractionated when this was necessary, and were obtained from J. Burrough Ltd., May and Baker Ltd., and British Drug Houses Ltd.

Ion Exchange Resins: Amberlite IRC - 50 mesh 72 - 100) and "Analytical Grade" Amberlite IRA - 400 (mesh 50 - 100) were used. The resins were obtained in the required mesh

sizes by grinding and sieving the materials.

Water: Distilled water from a stoneware tank fed from a stainless-steel still was used. The water was free from copper.

INVESTIGATIONS OF THE STRONTIUM AND BARIUM COMPLEXES OF 8-HYDROXYQUINOLINE

If these complexes were formed over different pH ranges and were soluble in polar solvents, it was hoped that a separation of strontium and barium might be achieved on ion exchange resin columns.

Consider a complexing agent CH which forms soluble complexes CM_1 and CM_2 with the metal chlorides M_1Cl and M_2Cl. If CM_1 forms completely over, say, a pH range 2 - 6 and CM_2 over a pH range 6 - 10, then a complete separation of the metals could be achieved by eluting one, from the column, with a solution of the complexing agent at pH4 or the other, similarily, at pH8. For example, aluminium and magnesium form complexes with 8-hydroxyquinoline. Since the pH ranges of complete complex formation are aluminium 4.2 upwards and magnesium 8.2 upwards[18], it should be theoretically possible to separate these metals by taking them on to a cation exchange resin and eluting at pH5 with an aqueous-organic solution of 8-hydroxyquinoline, which will dissolve the aluminium complex.

Magnesium and the alkaline earths form complexes with 8-hydroxyquinoline over different pH ranges. The pH values at which

precipitation starts and is complete were found by Näsänen[19] and are given in Table 1.

TABLE 1

Metal	Precipitation starts	Precipitation complete
Magnesium	5.6	6.5
Calcium	7.8	9.0
Strontium	8.7	10.0
Barium	9.2	>11.0

These values apply for a metal concentration of 0.05 g. ions/litre and an 8-hydroxyquinoline concentration of 3.78×10^{-3} g. mol./litre. Näsänen emphasises, however, that the pH values are highly dependent on the amount of reagent used.

Investigations of the complexes of strontium and barium with 8-hydroxyquinoline were undertaken to see if favourable conditions existed for their use in separating these elements on a resin column. Certain criteria had to hold if there was to be any possible chance of a separation being effected. They were (1) that the pH ranges over which formation was complete were not the same, (2) that the solubility of the complexing agent in the eluting solvent should exceed 1%, (3) that the solubility of the complex in the solvent should

exceed 0.1%, and (4) that the solvent should be polar so that ionisation of the reactants could occur.

As mentioned above, strontium and barium do form complexes with 8-hydroxyquinoline over somewhat different pH ranges, but, despite a wide search no suitable organic solvent could be found to dissolve the water - insoluble complexes formed between 8-hydroxyquinoline and solutions of strontium and barium nitrates. These investigations were therefore terminated.

Dyrssen[20] has since found that strontium can be extracted from an aqueous solution into a solution of 8-hydroxyquinoline in chloroform if the concentration of the organic solute is high, but it is doubtful if this method could be applied to the removal of strontium from ion exchange resins.

Papers have recently appeared on the separation on ion exchange columns of magnesium-calcium with hydrochloric acid[21], calcium-strontium-barium with ammonium lactate[22], strontium-barium with ethylene diamine tetra-acetate[23] and magnesium-calcium-strontium-barium with ammonium citrate as eluting agent[24].

The effect of the addition of acid, alkali and chlorides on the absorption of a 1% solution of 8-hydroxyquinoline in acetone - water (75 : 25% V/V).

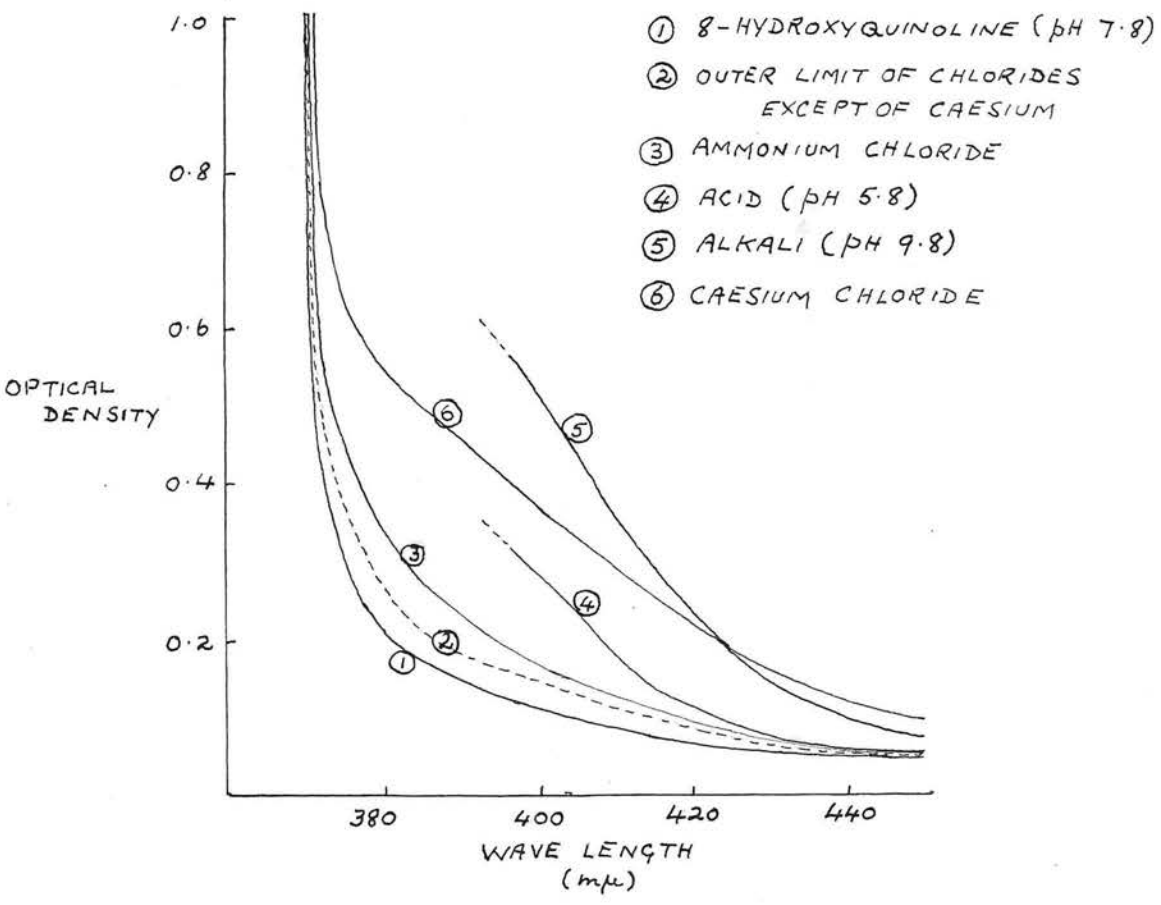


FIGURE 2.

INVESTIGATIONS ON THE REACTION OF CAESIUM CHLORIDE WITH 8-HYDROXYQUINOLINE

When caesium chloride is added to an aqueous-acetone solution of 8-hydroxyquinoline, there is a marked deepening in colour of the solution and this does not occur with the other alkali metal chlorides.

8-Hydroxyquinoline itself is practically insoluble in water but a 1% W/V solution may be made in acetone-water (3 vol. : 1 vol.) or alcohol-water (3 vol. : 1 vol.). The former solution is almost colourless and has a pH of 7.8. The colour of the solution deepens to pale yellow on adding acid or alkali. Solutions of pH 5.8 and 9.8 were prepared by adding 0.05 ml. of 2N acetic acid and 0.05 ml. of 2N ammonia respectively, to 15 ml. of the 1% solution. The absorption of each of these solutions was measured as a function of wave length on the Unicam SP 500 spectrophotometer. The curves are shown in Figure 2.

Half the equivalent amounts of ammonium chloride and the chlorides of lithium, sodium, potassium, rubidium and caesium were added to 5 ml. of the solution at pH 7.8. A deepening in colour was only observed for the solution containing caesium chloride. The absorption curves for these solutions are also shown in Figure 2.

The curves for the solutions containing the chlorides of lithium, sodium, potassium and rubidium lie very close to the 8-hydroxyquinoline curve while those due to ammonium chloride and caesium chloride are displaced, the former slightly and the latter to a greater extent. The displacement of the ammonium chloride curve is probably due to the fact that the addition of this salt to the 1% solution caused a lowering in pH and hence a greater absorption. Caesium chloride is neutral in aqueous solution, however, and the displacement of the caesium chloride curve can only be explained by salt or complex formation with the 8-hydroxyquinoline. Attempts were made to utilise this difference in the reactions of the rubidium and caesium ions in order to obtain a separation of these two elements. A solution of 8-hydroxyquinoline in alcohol-water was preferred to one in acetone-water as the former solvent is more polar.

Attempted separation on an ion exchange resin column

A strong cation exchanger such as Dowex 50 could not be used since it would hold the alkali metal ions too strongly. Amberlite IRC -50 a weak cation exchanger whose functional groups consist entirely of carboxyl groups was considered

suitable. Since 8-hydroxyquinoline is not dissociated to any large extent at pH 7.8 (the acid dissociation constants in 50% dioxane at 25°C are pK_{NH} 3.97 and pK_{OH} 11.54[25]), it was considered advisable to add a substance, which was itself completely dissociated, and which, in the absence of the organic compound, would remove rubidium and caesium very slowly. Ammonium chloride was chosen for this purpose and the resin was worked in the ammonium form. Removing the caesium first by means of ammonium ion, would yield free caesium ions in the solution and so assist in the formation of caesium 8-hydroxyquinolinate.

The following technique was adopted. A column of Amberlite IRC - 50 was prepared and put through several cycles by changing to the hydrogen (2N hydrochloric acid) and ammonium (2N ammonium chloride in 2N ammonia) forms alternately. The column in the ammonium form was washed with very dilute ammonia at pH 7.8 until free from chloride ion. A mixture of rubidium and caesium chlorides containing rubidium 86 and caesium 134 isotopes, was taken on to the column which was again washed with very dilute ammonia followed by a solution of alcohol-water (3 : 1 V/V) at pH 7.8. A solution

of ammonium chloride of the correct concentration in alcohol-water (3 : 1 V/V) at pH 7.8 and 1% W/V with respect to 8-hydroxyquinoline was run through the column slowly. It was hoped that caesium would be removed before rubidium.

Trials to find the correct ammonium chloride concentration were made on a 20 cm. x 1 cm. diameter column of Amberlite IRC - 50 in the ammonium form using rubidium chloride (4 mg. cation) containing the radioactive rubidium 86 isotope. A 5% W/V solution of ammonium chloride in alcohol-water (3 : 1 V/V) at pH 7.8 removed the rubidium from the column as soon as the solution in the resin bed had been replaced. No rubidium was removed from the column in the first 80 ml. of effluent collected at 1 ml./sq.cm./min., when a 0.5% W/V solution of ammonium chloride in alcohol-water (3 : 1 V/V) at pH 7.8 was used, but, by passing the column under a G.M.4 solid counter it was ascertained that the rubidium front had travelled a distance of about 14 cm. From these investigations it appeared that a 0.5% W/V solution of ammonium chloride might satisfy the required conditions for a separation.

A run with caesium chloride (4 mg. cation) tagged with caesium 134, was made on the same

resin column. Alcohol-water (3 : 1 V/V) containing 0.5% W/V ammonium chloride and 1% W/V 8-hydroxyquinoline was used at pH 7.8. The effluent was again collected at 1 ml./sq.cm./min. No caesium was removed in the first 112 ml. of solution. It therefore appeared that no strong complex formation was occurring with the 8-hydroxyquinoline. If the caesium ion had been bound firmly in a complex then it would no longer have been available for re-exchange on to the resin and would have been completely removed from the column leaving the rubidium behind.

Although the results of the preliminary investigations, indicated that rubidium and caesium might be separated on an ion exchange resin column, it was now not considered worth while continuing, since, in the absence of strong complex formation between caesium and 8-hydroxyquinoline, a large volume of eluant at a slow rate of elution would be required for a separation, if it could be achieved at all.

The caesium ion does however appear to have some affinity for the phenolic group, for it has been found[26] that caesium chloride in the presence of Amberlite IR - 100 resin, shows two adsorption peaks at pH 4 and 6. The first

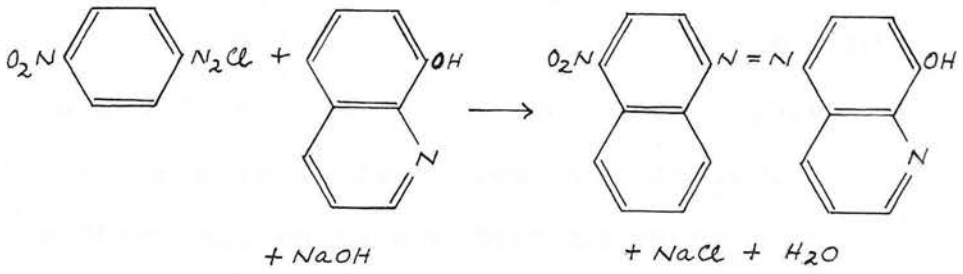
is attributed to - SO_3Cs and the second to - OCs. Caesium is selectively adsorbed by the phenolic groups in the presence of high concentrations of sodium salts in alkaline solutions.

Attempts to obtain a colorimetric method for determining caesium.

As no success had been achieved by using 8-hydroxyquinoline in attempts to separate rubidium and caesium on a resin column, there remained a possibility of exploiting the colour change obtained when caesium chloride is added to an 8-hydroxyquinoline solution, to give a colorimetric method for the determination of caesium. The slight change in colour observed on adding caesium chloride was not sufficient to make a direct method possible and so it was decided to try an indirect method.

It was assumed that the increase in colour was due to the water soluble caesium 8-hydroxyquinolinate being formed. A solution of caesium chloride was shaken up with a 1% W/V solution of 8-hydroxyquinoline in carbon tetrachloride and the complex formed. The uncombined 8-hydroxyquinoline was extracted from the aqueous layer with carbon tetrachloride and the aqueous layer made definitely alkaline with dilute sodium hydroxide solution. To

this solution a diazotised solution of p-nitraniline in dilute hydrochloric acid was added, making sure that an excess of alkali was present in the final solution. A red dye should have been produced.



1 ml. of a 1% W/V aqueous solution of caesium chloride (10 mg. cation) was diluted to 5 ml. and shaken up with 10 ml. of a 1% W/V solution of 8-hydroxyquinoline in carbon tetrachloride. The tetrachloride layer was separated from the aqueous layer which was then twice shaken up with 5 ml. portions of tetrachloride to extract uncombined 8-hydroxyquinoline. 4 ml. of the aqueous layer were pipetted into a 25 ml. graduated flask and 2 ml. dilute sodium hydroxide added. The solution was cooled in ice-cold water. To 5 ml. of a well-cooled saturated solution of p-nitraniline in dilute hydrochloric acid, drops of an ice-cold 10% W/V aqueous solution of sodium nitrite were added with stirring until a definite excess of nitrous acid was present

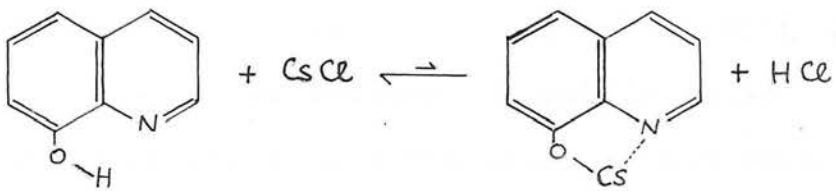
(immediate reaction with starch iodide paper). 1 ml. of this solution was then pipetted into the flask and the solution made up to the mark with water. No red dye was formed, but only a yellow-green solution. A blank (4 ml. water) also gave a yellow-green solution, while 4 ml. of a saturated aqueous solution of 8-hydroxyquinoline gave a bright red colouration. It was therefore concluded that no caesium 8-hydroxyquinolinate was present in the aqueous layer before treatment with p-nitrobenzene diazonium chloride solution.

Only two things could have happened, either (1) the complex formation is weak and probably not complete so that on shaking with carbon tetrachloride, it is broken down and the 8-hydroxyquinoline completely extracted, or (2) the complex is soluble in carbon tetrachloride and is also extracted from the aqueous layer.

This second possibility was disproved by means of a radioactive tracer method. 5 ml. of an aqueous solution of caesium chloride containing 10 mg. caesium ion and 9,000 counts/min. activity was treated with the 8-hydroxyquinoline, etc. as above. The final aqueous layer and combined carbon tetrachloride layers were tested for activity. All the

activity was present in the aqueous layer and no complex had been extracted by carbon tetrachloride.

These results prove that, if the increase in colour on adding caesium chloride to a 1% W/V solution of 8-hydroxyquinoline in acetone - water (3 : 1 V/V) is due to complex formation, only a weak complex can have been formed to the extent of probably no more than a few per cent. It is probable that an equilibrium exists as follows.



On extracting uncombined 8-hydroxyquinoline with carbon tetrachloride, the complex on the right breaks down to form more 8-hydroxy-quinoline which is removed on the next extraction.

It was therefore concluded that no colorimetric method for determining caesium could result from investigations of caesium salts in 8-hydroxyquinoline solution.

THE SEPARATION OF BERYLLIUM, MAGNESIUM AND THE ALKALINE EARTHS BY PAPER CHROMATOGRAPHY

In partition chromatography, the choice of a solvent-mixture is one of the major, if not the major consideration. Some inorganic metal salts are very soluble in organic solvents and can, therefore, be run away from less soluble material. Generally, however, it is desirable to develop a solvent-mixture which has a small, though finite solvent action on the salts. By this means, redistribution will occur many times between the mobile phase (solvent-mixture) and the water in the paper (stationary phase) as the solvent-mixture moves down the paper, so that, with only slightly different partition coefficients, the repeated partitioning will bring about distinct separations. Many metals can be separated by this simple procedure.

For some metal salts, however, the use of a simple solvent is not adequate. The salts of magnesium, calcium, strontium and barium and potassium, rubidium and caesium, may be cited as examples. It is almost impossible to achieve satisfactory separations of these elements by the simple procedure just

considered. It is necessary, therefore, to develop other means to alter the partition coefficients to facilitate separations. Among the possibilities may be listed, (1) the addition to the solvent-mixture of a strong acid corresponding to the anion in the original salt, (2) the addition of a complex-forming reagent which will convert the original salt into a complex of the metal ion (the formation of the complex depending upon the equilibrium, metal ion + complexing agent = metal complex + hydrogen ion, and hence being dependant upon the pH of the solvent-mixture), and (3) the preparation and use of salts of the metals containing organic anions. Salts of this type generally show a wider variety of solubility in organic solvents.

Sufficient information was known about (1), to indicate that further improvement in the method of Miller and Magee[4] was unlikely and therefore, it was hoped that (2) or (3), or a combination of both, might be successful.

Preliminary Investigations

A review of the chemical literature indicated that the solvent-mixture, developed by Pollard and co-workers[7], was probably the most successful for the separation of magnesium, calcium, strontium and barium.

This solvent-mixture consists of equal volumes of symm. collidine and 0.4 N nitric acid and the R_F values magnesium 0.65, calcium 0.52, strontium 0.40 and barium 0.26 are given. When chromatograms were run with this solvent-mixture the solvent front travelled 25 cm. in 16 hr. on Whatman No. 1 paper. A mixture of 50 µg. amounts of each cation (as nitrates), placed on the starting line as a spot, was not completely resolved, since magnesium-calcium and strontium-barium were not fully separated. The R_F values of the mid-point of each spot were barium 0.16, strontium 0.29, calcium 0.45 and magnesium 0.57, with the acid front at 0.73. A better separation than this was aimed at.

With the exception of Erlenmeyer and co-workers[8], who used acetates, previous workers had employed salts of inorganic acids. In this research, n-butyrates were employed since it was already known that the butyrates of certain elements are soluble in organic solvents[27]. Sandaram and Banerjee[28] had also reported that beryllium may be extracted into chloroform from an aqueous solution of beryllium chloride containing butyric acid.

Preparation of n-Butyrates: These were first made by dissolving the carbonates in equal volumes of water and n-butyric acid, but there appeared to be no easy way of removing excess acid and they were later prepared by ion exchange. For this purpose a 20 cm. x 1 cm. diameter column of Amberlite IRA - 400 (10 gm. of mesh 50 - 100) was used to change 20 mg. amounts of the cations, present as chlorides or sulphates, to butyrates. 200 ml. of 2N sodium hydroxide regenerated the resin completely to the hydroxyl form. After washing with an equal volume of water, 50 ml. of 2N butyric acid were passed through and after a final washing with 300 ml. water, the column was ready for use. The appropriate salt, dissolved in a small volume of water, was taken on to the column and washed through with water. 50 ml. portions were collected (70 ml. for beryllium) and the solutions gently evaporated down to 2 ml. first in small beakers and then in small tubes immersed in boiling water. Water free from carbon dioxide was used throughout. The solutions then contained 1% W/V of cations.

Beryllium butyrate could not be prepared by the above method and was not used. The

effluent from the column was quite clear but, on boiling the solution, the butyrate or basic butyrate was hydrolysed and beryllium hydroxide precipitated.

Solubilities of n-Butyrates: The solubilities of the butyrates of magnesium and the alkaline earths were investigated. It was found that they are soluble in water, anhydrous butyric acid and methyl alcohol. They are slightly soluble in higher alcohols, the solubility decreasing as the carbon chain lengthens. They are insoluble in ethyl butyrate, ether, acetone, chloroform, carbon tetrachloride and benzene.

Solvent-mixtures: Single alcohols and mixtures of alcohols were first used with Whatman No. 1 paper. Methanol - n-butanol (50 : 50% V/V) and ethanol were the most satisfactory solvents but extensive tailing occurred. No improvement in the tailing resulted when up to 20% of water was included in the solvent-mixtures. The tailing was reduced on adding 20% of aqueous butyric acid to the solvent-mixtures. In order to find the optimum normality for the butyric acid, strips were run with methanol - n-butanol - n-butyric acid (40 : 40 : 20% V/V) at different normalities of the acid, viz. 1, 2, 4, 6 and 8N. The least

TABLE II

Effect of some solvent-mixtures on the Group IIA butyrates.

No.	Solvent Mixture	Amount of each ion (µg.)	R_F Values			
			Magnesium	Calcium	Strontium	Barium
1.	Methanol-Butanol (50 : 50% V/V)	200	0.66 - 0.84	0.55 - 0.73	0.0 - 0.36	0.0 - 0.17
2.	Ethanol	200	0.65 - 0.90	0.15 - 0.72	0.0 - 0.37	0.0 - 0.13
3.	Methanol-Butanol-2N butyric acid (40 : 40 : 20% V/V)	200	0.69 - 0.82	0.62 - 0.75	0.24 - 0.56	0.03 - 0.43
4.	Ethanol-6N butyric acid (80 : 20% V/V)	50	-	0.54 - 0.72	0.16 - 0.47	0.03 - 0.33
5.	Methanol-Butanol-6N butyric acid (40 : 40 : 20% V/V)	200	0.67 - 0.77	0.65 - 0.76	0.24 - 0.58	0.01 - 0.37

tailing occurred with 6N acid. The bands obtained with ethanol - 6N butyric acid (80 : 20% V/V) were inferior to those obtained with methanol - n-butanol - 6N butyric acid (40 : 40 : 20% V/V). Table II gives the R_F values obtained with a selection of the various solvents employed.

Solvent-mixture No. 5 separated calcium, strontium and barium, in amounts up to 50 µg. but not magnesium and calcium. The R_F values are measured from the front and back of the bands. The minimum amounts of calcium, strontium and barium detectable were 10 µg.

Next, the influence of a complexing agent was investigated. By this means, it was hoped to influence the partitioning of the salts in the solvent-mixture and achieve a better separation. The base symm. collidine was found to be effective and added to solvent-mixture No. 5 gave a marked improvement in banding. The solvent-mixture used was methanol - n-butanol - 6N butyric acid - symm. collidine (40 : 20 : 20 : 20% V/V). A mixture of 100 µg. amounts of calcium, strontium and barium were well separated but 50 µg. amounts of magnesium and calcium still touched. The R_F values for a mixture of 50 µg. of each of the

cations were, barium (0.11 - 0.26), strontium (0.30 - 0.47), calcium (0.64 - 0.71) and magnesium (0.71 - 0.81). Further investigations with the nitrates, chlorides and acetates of the metals showed that they gave similar R_F values to the butyrates and, since they were more readily prepared, were clearly more advantageous to use. However, when nitrates were used, it was found that a band due to collidinium nitrate interfered by coinciding with the magnesium and calcium positions. With the discovery that other salts could be used, butyric acid in the solvent-mixture was replaced by acetic acid and the following composition used, methanol - n-butanol- symm. collidine - 6N acetic acid (40 : 20 : 20 : 20% V/V). 50 µg. amounts of magnesium, calcium, strontium and barium were completely separated in a 35 cm. run, the band due to collidinium nitrate now falling below the magnesium position and causing no interference. This solvent-mixture was adopted for all future work with the five metals in the chloride form. The R_F values of Group IIA cations and other elements with the solvent-mixture: These were obtained on Whatman No. 1 paper and are given below in Table III. Chlorides and nitrates

were used and the solutions, spotted on to the paper, were in dilute hydrochloric or nitric acid if there was a likelihood of hydrolysis occurring in aqueous solution. These values refer to 50 µg. amounts of Group IIA cations and 100 µg. amounts of others in 0.01 ml. solution.

TABLE III

Element	Spraying Reagent	R_F Values
Be	8-Hydroxyquinoline	0.85±0.06
Mg	"	0.53±0.05
Ca	"	0.41±0.04
Sr	Sodium rhodizonate	0.21±0.05
Ba	"	0.09±0.05
Ag	p-Dimethylamino benzylidine rhodanine in acetone	0.93±0.03
Hg^{I}	"	0.90±0.05
Pb	Sodium rhodizonate	0.55±0.05
Cu	Cupferron in methanol	0.83±0.05
Cd	Diphenylcarbazide in ethanol	0.76±0.19
Hg^{II}	"	0.88±0.07
As^{III}	Diphenylthiocarbazone in chloroform	Unsatisfactory
Sb^{III}	"	"
Sn^{IV}	"	"
Bi	Aq. solution of sodium sulphide	"
Fe^{III}	Cupferron in methanol	0.91±0.07

Element	Spraying Reagent	R_F Values
Cr	Hydrogen peroxide followed by diphenylcarbazide in alcohol	0.88±0.08
Al	8-Hydroxyquinoline	0.88±0.10
Mn	Saturated aq. potassium periodate	0.66±0.04
Zn	8-Hydroxyquinoline	0.89±0.05
Co	1-nitroso 2-naphthol in methanol	0.81±0.07
Ni	Sodium salt of dimethyl-glyoxine	0.83±0.07
Li	Zinc uranyl acetate	0.52±0.06
Na	"	0.22±0.06
K	Sodium lead cobaltous hexanitrite	0.08±0.06
NH_4	"	0.30±0.07

Since the solvent-mixture is neutral, extensive or complete hydrolysis occurred with bismuth, arsenic, antimony and tin. Iron, aluminium and chromium salts were slightly hydrolysed, giving a faint spot at the start and a faint tailing backwards.

Choice of cellulose medium: When the final composition of the solvent-mixture was decided, a switch was made from Whatman No. 1 to Whatman 3MM paper which was preferred because it could accommodate a greater weight of cations, a fact considered to be of value when the method

came to be applied to the analysis of carbonate rocks and other materials.

Development of Spraying Reagents: The following compounds were investigated, viz. sodium rhodizonate, 8-hydroxyquinoline, violuric acid, 2-thiovioluric acid, chloranilic acid, solochrome black, murexide, kojic acid, alizarin and quinalizarin. The first two were the only reagents considered to be sensitive enough and satisfactory for detecting the positions of the cations.

A saturated aqueous solution of sodium rhodizonate was used for detecting strontium and barium. These cations form red precipitates with the reagent.

A 5% W/V solution of 8-hydroxyquinoline in methanol - chloroform - water (85 : 10 : 5% V/V) was used to detect beryllium, magnesium and calcium. This reagent had been employed by Miller and Magee[4]. Formation of the 8-hydroxyquinolinates can be brought about by hanging the strips for a few minutes in a jar containing a beaker of concentrated ammonia. Under these conditions, the Group IIA precipitates fluoresce in ultra-violet light; beryllium and magnesium-yellow and calcium-green. Strontium and barium show only weak

fluorescence. With Group IA elements only lithium shows a fluorescence which is bright blue. The solvent-mixture was developed for the separation of the Group IIA elements in the presence of the alkali metal cations and the fluorescence due to lithium interferes with the detection of magnesium and calcium. However, lithium 8-hydroxyquinolinate is only precipitated at a high pH and is not formed when the papers are sprayed with this reagent containing 0.1N sodium hydroxide solution (carbonate-free) in the proportions, 0.1 ml. alkali to 5 ml. 8-hydroxyquinoline solution. Under these conditions, beryllium, magnesium and calcium fluoresce brilliantly, while strontium and barium show no fluorescence at all.

These conditions apply only when a weak acid, weak base or weak acid + weak base are used in the solvent-mixture. When a strong acid e.g. nitric acid is included in the mixture the addition of more alkali is necessary for the full development of the colours. It is essential that the 0.1N sodium hydroxide should be added to the 8-hydroxyquinoline solution immediately before spraying. Otherwise, changes occur in the organic reagent and inferior colours are obtained. Spraying a paper with

8-hydroxyquinoline reagent does not effect the sensitivity of the sodium rhodizonate for strontium and barium.

Detailed Procedure

1. Place the solvent-mixture, methanol - n-butanol - symm. collidine - 6N acetic acid (40 : 20 : 20 : 20 % V/V) in the solvent trough of the Shandon Chromatank at least one hour before the experiment is started.

2. On a Whatman No. 3MM filter strip, 16 cm. wide and 57 cm. long, draw pencil lines, 1, 5, 6 and 7 cm. from the top end of the strip and fold the paper along the first three of these lines, so that, when the upper end dips into the solvent trough, the length from the 7 cm. mark to the end hangs vertically and gives a solvent run of 50 cm.

3. Place 0.01 ml. of the test solution as a spot on the paper along the 7 cm. line. Four such spots may be accommodated by placing them 4 cm. apart across the paper.

4. Allow the spots to dry and insert the strip in the chromatographic vessel. Allow the solvent-mixture to run down the paper for a distance of 45 to 50 cm.; this usually requires 16 to 18 hours and it is very suitable to leave the paper overnight.

5. Remove the paper from the apparatus and hang it in the air to dry for about 30 minutes; next, heat it in an oven at 110°C until very little smell of the solvent-mixture persists. The paper is now ready for spraying.

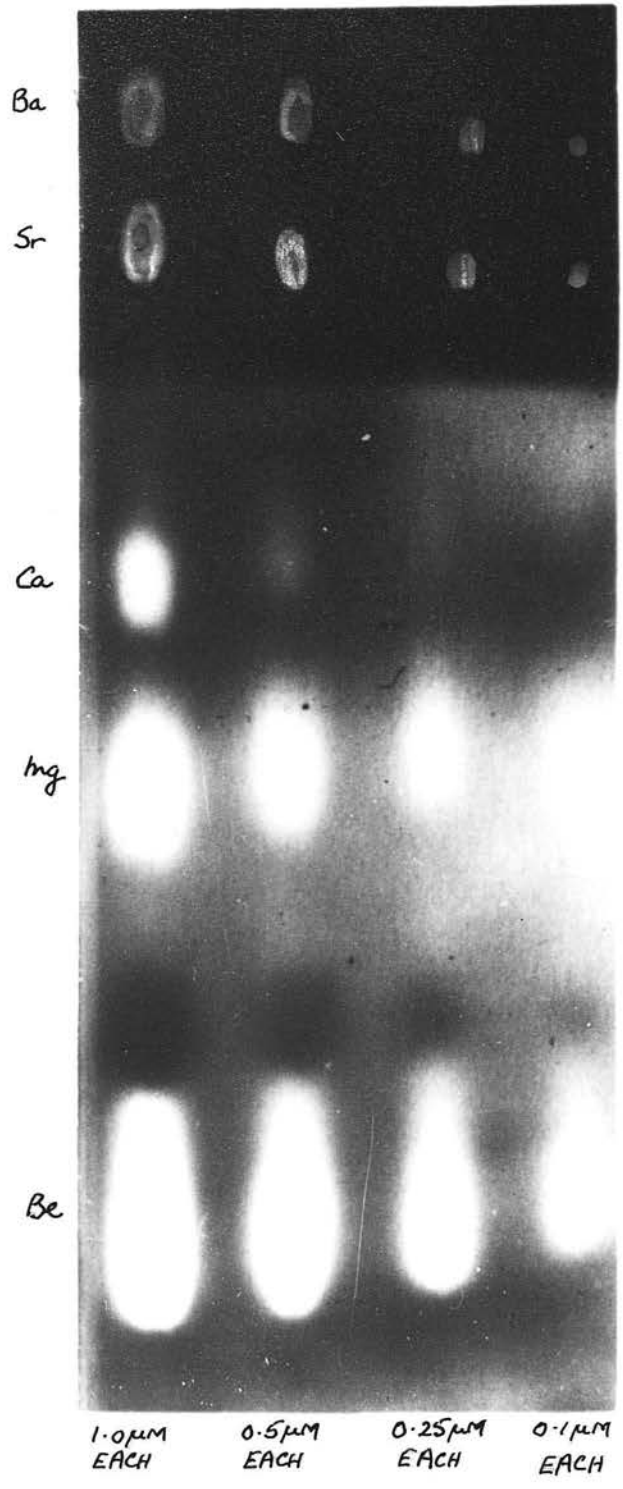
6. Spray the paper with 8-hydroxyquinoline reagent adjusted in the described manner. Allow the paper to hang in the air for 15 minutes to ensure complete drying and examine it under an ultra-violet lamp. Under these conditions, beryllium, magnesium and calcium fluoresce brilliantly. For estimation purposes compare the spots with a set of standards. The areas and intensities of the spots increase with increasing amounts of the elements.

7. Spray the upper portion of the paper with the sodium rhodizonate reagent. Allow it to dry completely and compare with standards as before.

Standards, Limits and Synthetic Mixtures

Since five cations with a range of atomic weights 9(Be) to 137(Ba) were being investigated, all quantities were now expressed throughout in micromoles (μM) in preference to microgram (μg.) amounts. Using the solvent-mixture, methanol - n-butanol - 6N acetic acid - symm.

PLATE 1.



collidine (40 : 20 : 20 : 20% V/V), standards were run on Whatman 3MM paper with 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 μM amounts of the Group IIA elements in 0.01 ml. solution. The 0.1, 0.25 and 0.5 and 1.0 μM standards are shown in Plate 1. The beryllium, magnesium and calcium spots were photographed in ultra-violet light, the strontium and barium spots in daylight. Limits were also run with beryllium 10 μM others 0.1 μM, magnesium 10 μM others 0.1 μM, etc., since it was intended to separate and detect, if possible, a total of 10 μM of cations where the minimum amount of any constituent was 0.1 μM. The standards and limits were satisfactory except for large and very small amounts of calcium. With calcium $\geq$ 5 μM there is a tendency for tailing to occur in the calcium spot. The spots due to other cations when present with five or more micromoles of calcium are also inferior to those usually obtained.

In the procedure used, 0.01 ml. volumes of solution are spotted on to the paper and the solvent allowed to evaporate before placing the paper in the chromatographic vessel. Presumably calcium would be present on the paper,

as hydrated calcium chloride. In the test-tube, hydrated calcium chloride crystals give a dense white gelatinous precipitate with the solvent-mixture. This precipitate is very soluble in water but not readily soluble in excess of the solvent-mixture and is probably a calcium chloride-collidine complex. Hydrated magnesium chloride crystals dissolve but hydrated strontium chloride and barium chloride crystals give precipitates under similar conditions, not however so readily as calcium chloride. It would therefore appear that, with large amounts of calcium, precipitation of a complex occurs on the paper (indeed this has been noticed on several occasions) and tailing results. Other cations could be occluded in the precipitate and tailing of them also occur. Running chromatograms before allowing the spots to dry out, helps but does not eliminate the trouble.

Whatman 3MM paper contains a trace of calcium and all sprayed chromatograms show a faint fluorescent band across the paper in the calcium position. Because of this, the detection of 0.1 μM amounts of calcium was uncertain. 0.1 μM of calcium can however be detected with ease when a mixture is separated

TABLE IV

Analysis of 'unknown' mixtures. Estimated amounts (μM) of metals found.

No.	Barium	Strontium	Calcium	Magnesium	Beryllium
1	5(4)	0	0.25(0.1)	2(5)	0.2
2	8	0.3 (0.1)	0.1	0.5(1)	0
3	0	0.4 (0.1)	8	0	1
4	0	0	0.5 (0.2)	0.25(0.1)	8 (9)
5	0	0.25(0.1)	0 (0.1)	2.5 (9)	0.1
6	1	1 (0.5)	0.1 (0.2)	2.5 (8)	0
7	0.35(0.2)	10(9)	0.5 (0.1)	0	0
8	0.8 (0.5)	0.25(0.1)	5 (8)	0 (0.1)	0.25(0.5)
9	0.4 (0.5)	0 (0.1)	8 (10)	0	0
10	0	0.25(0.1)	0.4 (0.25)	7 (10)	0
11	0	0	0.35(0.1)	0.1	9 (10)
12	0.2 (0.1)	0.25(0.1)	5	0.25(0.1)	4 (3)
13	0.4 (0.2)	0.35(0.2)	8 (7)	0.1	0
14	0	0	0.4 (0.25)	10	0

on the double-acid washed Whatman No. 41 paper. With this paper no fluorescent calcium background is obtained.

The R_F values of 1 μM amounts of the five cations are barium 0.05 to 0.15, strontium 0.17 to 0.27, calcium 0.44 to 0.51, magnesium 0.55 to 0.64 and beryllium 0.78 to 0.93.

As a final test of the validity of the proposed scheme, 14 mixtures of 'unknown' composition were analysed. The results are shown in Table IV and are the estimated amounts in micromoles of the metals found. Where these differ from the amounts present, the latter are shown in parenthesis.

In mixture No. 5 doubt was felt about the presence of the minimum amount of calcium. Because of this doubt it was reported as missed. In mixtures No. 8 and No. 9, both of which contained more than 5 μM of calcium, the 0.1 μM amounts of magnesium and strontium respectively were missed. The reason for failing to detect these minimum amounts has already been stated (p. 41). The results are considered to be satisfactory.

Application of Solvent-mixture in Qualitative Analysis

The Groups IA and IIA salts in extracts from grasses and clovers have been separated by the solvent-mixture and magnesium, calcium and potassium detected (potassium with sodium lead cobaltous hexanitrite reagent, see p. 58). A dolomite, a strontianite and a barytocalcite have also been examined for magnesium and the alkaline earths. It is not necessary to separate iron, aluminium, etc., from the solution before running a chromatogram since all the elements likely to be present in a carbonate rock are moved down towards the solvent front. However, since the solvent-mixture is neutral, interference from phosphate would probably occur if there was not sufficient iron and aluminium to bind it.

The procedure was incorporated into the qualitative scheme of analysis used by third year students in the University of Edinburgh, during the last two sessions. For the detection and estimation of the elements concerned, namely, barium, strontium, calcium and magnesium, it proved to be very successful.

The solvent-mixture should be useful in the qualitative analysis with rough estimations

of the Group IIA metals in mineral waters, soil samples, carbonate rocks, etc. The method has the advantage of requiring only very small amounts of materials, viz. 0.01 ml. of solution containing never more than 1.5 mg. of cations and usually a much smaller quantity. It should be particularly suited for the analysis of single crystals in igneous rocks and the small inorganic residues obtained on evaporating and ashing the extracts from grasses and leaves. The application of this procedure to quantitative determinations in natural materials is reported and discussed later in the thesis.

During the course of this work and since it has been completed, further separations of certain of the Group IIA elements have been reported[29], [30], [31], [32]. The most interesting of these is by Tristram and Phillips[31] who, after separating the chlorides of sodium, potassium, magnesium and calcium, go on to determine the separated salts by a volumetric technique.

THE SEPARATION OF ALKALI AND AMMONIUM SALTS BY PAPER CHROMATOGRAPHY.

As already mentioned in the introduction, the separation of lithium, sodium and potassium is readily accomplished but the separation of potassium, rubidium and caesium is much more difficult. A separation of the alkali metals on one strip was aimed at, as it was later hoped to determine the amount of each of the separated salts by micro-analytical methods.

Preliminary Investigations

Although the acetates[8] and citrates[33] of lithium, sodium and potassium have been separated, most of the previous investigators used the salts of inorganic acids. The separation of the alkali metal salts of higher aliphatic acids has not been studied and therefore, as with the Group IIA metals, n-butyrates were first employed to investigate the separation of potassium, rubidium and caesium with solvent-mixtures of organic compounds. The later work was done with chlorides.

Preparation of n-Butyrates: These were prepared by ion exchange. For this purpose a column (10 cm. x 1 cm. diameter) of Amberlite IRA - 400

(5 gm. of resin, mesh 50 - 100) was used to change 20 mg. amounts of lithium, sodium, potassium, rubidium, caesium and ammonium ions as chlorides to butyrates. The following technique was adopted. The resin was regenerated with 100 ml. of 2N sodium hydroxide solution and washed with 100 ml. of water. It was changed to the butyrate form with 25 ml. of 2N butyric acid at a flow rate of 2 ml./min. and washed with 200 ml. of water. The salt was dissolved in a little water and taken on to the column. 30 ml. of solution were collected at 1 ml./min. and evaporated down to a small volume in a beaker by gentle boiling. The solution was transferred to a small tube and evaporated to 2 ml. by immersing the tube in boiling water, using, as an aid, a fine jet of air. All the water was free of carbon dioxide. The solutions had concentrations of approximately 100 µg. metal ion per 0.01 ml.

Solubilities of the n-Butyrates: With the commonly used solvents, the alkali metal butyrates are readily soluble only in water, methyl alcohol and anhydrous butyric acid.

TABLE V

No.	Solvent Mixture	Amount of each ion (µg.)	R_F Values				
			Lithium	Sodium	Potassium	Rubidium	Caesium
1.	Methanol	200	0.75 - 0.95	0.62 - 0.74	0.30 - 0.42	0.24 - 0.34	0.24 - 0.34
2.	Methanol - n-butanol (80 : 20% V/V)	200	0.85 - 0.97	0.46 - 0.58	0.16 - 0.30	0.10 - 0.22	0.06 - 0.18
3.	Methanol - n-butanol - water (70 : 20 : 10% V/V)	200	0.71 - 0.79	0.48 - 0.56	0.28 - 0.38	0.22 - 0.30	0.21 - 0.29
4.	Methanol - anhydrous butyric acid (80 : 20% V/V)	50	0.83 - 0.91	0.68 - 0.76	0.45 - 0.55	0.25 - 0.37	0.25 - 0.37

Solvent-mixtures:

Lithium, sodium and potassium butyrates were separated on Whatman No. 1 paper using certain mixtures of simple alcohols. A selection of these are shown in Table V.

No separations of potassium, rubidium and caesium were achieved with these solvent-mixtures of simple alcohols. Butyric acid was then introduced to the solvent-mixtures. Methanol-anhydrous butyric acid (80 : 20% V/V) was the most promising mixture, and, as shown in Table V, potassium and rubidium, but not rubidium and caesium were separated with it. From these chromatograms it was evident, however, that the butyrates were reacting in a similar manner to chlorides and other inorganic salts. Further investigations were made with chlorides.

Where solvent-mixtures of simple alcohols showed a slight tendency to a separation of potassium, rubidium and caesium ions, the order from the starting line was always caesium, rubidium and potassium. However, in the two previous references to a separation[4], [5], the order from the start was potassium, rubidium and caesium. It was therefore decided to use solvent-mixtures that would

TABLE VI

SOLVENT MIXTURES					R_F VALUES					
C. HCl	MeOH	BuOH	S.Coll.	Ketone	Sodium	Potassium	Rubidium	Caesium	Ammonium	Lithium
55	35	5	-	5	0.45	0.47	0.51	0.60	0.63	0.68
35	55	-	10	-	0.38	0.28	0.25	0.33	-	0.75
40	55	-	5	-	0.36	0.25	0.28	0.34	0.51	0.68
70	25	-	5	-	0.58	0.58	0.60	0.69	0.69	0.76
95	-	-	5	-	0.75	0.79	0.80	0.83	0.83	0.80
85	-	10	5	-	0.65	0.70	0.72	0.78	0.77	0.76
65	20	10	5	-	0.52	0.51	0.55	0.63	0.66	0.73

preserve this order, for preliminary investigations showed that a separation in the reverse order was unlikely.

Miller and Magee[4] used concentrated hydrochloric acid - methanol - n-butanol - methyl iso-butyl ketone (55 : 35 : 5 : 5% V/V) to separate potassium, rubidium and caesium. 100 µg. amounts of these cations, lithium, sodium and ammonium were chromatographed separately with this solvent-mixture on Whatman No. 1 paper and the R_F values for the mid-points of each spot are given at the top of Table VI. Sodium and potassium, ammonium and caesium were not separated. Chromatograms were prepared with a variety of other solvent-mixtures similar to that mentioned above. Most of these contained the organic base, symm. collidine and the R_F values of the elements are also shown in Table VI.

It can be seen that no separation of all six cations was achieved. In fact, no solvent-mixture would give a separation of potassium, rubidium, caesium and ammonium on one strip and so allow a mixture of these to be resolved and identified.

Steel[5] claimed a separation on Whatman No. 4 paper of 50 µg. amounts of ammonium,

potassium, rubidium and caesium ions with the phenol-rich layer obtained by shaking two volumes of 20% hydrochloric acid solution (i.e. 20 ml. concentrated diluted to 100 ml.) with one volume of molten phenol. The order of the separated salts from the starting line was ammonium, potassium, rubidium and caesium. Several runs were made with chlorides under similar conditions but Steel's results were never reproduced. Chromatograms run on Whatman Nos. 1 and 4 papers at 20°C in a constant temperature room, showed a good separation of 1 μM amounts of potassium, rubidium and caesium but the ammonium coincided with the rubidium position. Steel has however stated in a private communication that the flow of the ammonium ion is at times erratic and is dependant upon the anion present. The phenol-rich layers obtained when other proportions of dilute acid and phenol were used, gave almost identical chromatograms to those obtained with the above solvent-mixture. Lithium and sodium fall on and above the potassium position respectively.

In attempts to run lithium below potassium, rubidium and caesium, methanol was introduced to mixtures of phenol and concentrated

TABLE VII

No.	Solvent-mixtures			R_F Values					
				Li	Na	K	Rb	Cs	NH_4
1.	Phenol saturated with 20% hydrochloric acid			0.11	0.07	0.12	0.18	0.43	0.20
	Phenol	Methanol	C.HCl						
2.	33	33	33	0.55	0.24	0.21	0.28	0.42	0.42
3.	42	16	42	0.42	0.27	0.31	0.43	0.55	0.31
4.	20	30	50	0.70	0.44	0.43	0.50	0.64	0.64
5.	50	30	20	0.37	0.14	0.10	0.16	0.30	-
6.	25	50	25	0.57	0.19	0.11	0.14	0.23	-
7.	40	50	10	0.51	0.11	0.05	0.08	0.15	-
8.	50	40	10	0.37	0.06	0.04	0.06	0.13	-
9.	40	40	20	0.45	0.12	0.08	0.12	0.21	-
10.	Cresol-rich layer from m-cresol - c.HCl (90 : 10% V/V)*			0.09	0.01	0.04	0.09	0.31	-
11.	Catechol- MeOH - c.HCl (45 : 35 : 20% V/V)			Unsatisfactory chromatograms due to the very dark backgrounds produced with the spraying reagents.					
12.	Resorcinol - MeOH - c. HCl (50 : 30 : 20% V/V)			As 11					

* Whatman No. 4 paper was used.

hydrochloric acid. In the following work carried out with 1 μM amounts of each alkali cation on Whatman No. 1 paper, the methanol and acid are expressed in volumes (ml.) and the phenol as a weight (gm.). The relative positions of the cations with a number of these solvent-mixtures and others containing derivatives of phenol are given in Table VII. The R_F values refer to the mid-points of each band.

With a methanol content of 30 - 35% in solvent-mixtures containing phenol and concentrated hydrochloric acid (Nos. 2, 4 and 5) potassium, rubidium, caesium and lithium were separated in that order but the sodium could not be removed from the vicinity of the potassium. The separation of potassium and rubidium broke down if the methanol content exceeded 35%.

From these investigations it was evident that all the alkali metals and the ammonium ion would only be separated using two dimensional chromatography, with for example (1) methanol - n-butanol (70 : 30% V/V)[34] for the separation of potassium, sodium, ammonium and lithium and a solvent-mixture

consisting of phenol, methanol and concentrated hydrochloric acid for potassium, rubidium and caesium. However, for a detection and rough estimation, a solvent-mixture able to separate the groups potassium - rubidium - caesium - ammonium and lithium - sodium would be sufficient since selective spraying reagents could be used for the detection of the cations in each group.

Phenol - methanol - concentrated hydrochloric acid (50 : 30 : 20% W/V/V) will separate potassium, rubidium, caesium and lithium but ammonium lies on top of caesium. Phenol - methanol - concentrated hydrochloric acid (60 : 20 : 20% W/V/V) will separate potassium, rubidium, ammonium and caesium, but lithium partly overlaps ammonium which is only just clear of rubidium. The best spacing of potassium, rubidium, ammonium, caesium is given with phenol - methanol - concentrated hydrochloric acid (57.5 : 22.5 : 20% W/V/V) with which 1.7 µM amounts of the alkali and ammonium ions (10 µM total) in a run of 50 cm. on Whatman No. 1 paper give R_F values, potassium 0.08 - 0.14, sodium 0.09 - 0.13, rubidium 0.15 - 0.20, ammonium 0.23 - 0.27, lithium 0.27 - 0.32 and caesium 0.33 - 0.40. The separations are better on the

fast No. 4 paper and for later work with the alkali metals, Whatman No. 41 paper (double acid washed No. 4 paper) was used. This solvent-mixture gives superior spots to those obtained with the solvent-mixtures of Miller and Magee, and Steel.

Development of Spraying Reagents: The following compounds were investigated viz. zinc uranyl acetate, sodium lead cobaltous hexanitrite, sodium cobaltic hexanitrite, violuric acid and 2-thiovioluric acid. The first two reagents were the most satisfactory for detecting the positions of the alkali metals.

Zinc Uranyl Acetate - This reagent was prepared according to the method of Barber and Kolthoff[35]. Lithium and sodium ions are precipitated by this reagent as complex triple acetates which are detected by their fluorescence in ultra-violet light. The other alkali metals and the ammonium ion are not precipitated with the reagent.

Sodium Lead Cobaltous Hexanitrite - It is possible to have hexanitrites of Co^{II} and Co^{III}. The latter compounds are more stable and their formation is favoured if reducible substances are present in the solution. The cobaltic

hexanitrites are formed when excess nitrite is used in the presence of acid. Co^{II} is oxidised to Co^{III} while an equivalent amount of nitrous acid is reduced to nitric oxide.

$$Co^{II}Ac_2 + 2HAc + 7NaNO_2 \rightarrow Na_3 Co^{III}(NO_2)_6 + NO + H_2O + 4NaAc.$$

For the preparation of cobaltous hexanitrites it is essential to mix equivalent amounts of the compounds in the absence of free acid.

$$Co^{II}Ac_2 + PbAc_2 + 6NaNO_2 \rightarrow Na_2PbCo^{II}(NO_2)_6 + 4NaAc.$$

On standing, the $\left[Co^{II}(NO_2)_6\right]^{==}$ ion is slowly oxidised to $\left[Co^{III}(NO_2)_6\right]^{\equiv}$ and lead cobaltic hexanitrite $Pb_3\left[Co^{III}(NO_2)_6\right]_2$ starts to precipitate out. These effects are not always appreciated in methods for the preparation of cobaltous hexanitrites.

The cobaltous hexanitrite reagent is therefore prepared as follows. Dissolve 12.5 g.of cobaltous acetate tetrahydrate and 19.0 g. of lead acetate trihydrate in about 100 ml. of water. To this solution add a solution of 20.7 g. of sodium nitrite in about 100 ml. water. Mix thoroughly and dilute to 250 ml. Set aside for 1 hour and filter. The spraying reagent then

corresponds to the formula $Na_2PbCo^{II}(NO_2)_6$. This reagent is similar to that used by Sergienko[36] for the quantitative determination of potassium, but cobalt and lead acetates are substituted for cobalt and lead nitrates. On spraying, potassium and ammonium ions show up as grey spots, rubidium is brown and caesium appears as yellow-brown. Sodium and lithium ions give no precipitates with the reagent. Since ammonium lead cobaltous hexanitrite is the most difficult to precipitate of the four hexanitrite complex salts, the precipitation of amounts of ammonium ion less than 1 μM is assisted by spraying the strip with ethyl alcohol immediately before spraying with the reagent. After spraying, the strips should be left in the air for ten minutes until the spots are fully developed. The excess reagent, which itself produces a pale yellow background, is then washed off.

Potassium, rubidium, caesium and ammonium ions can also be revealed by spraying the strips with a solution of sodium cobaltic hexanitrite, $Na_3Co^{III}(NO_2)_6$, in dilute acetic acid. With, for example, excess potassium

ion, $K_3 Co^{III}(NO_2)_6$ is formed and with excess $[Co^{III}(NO_2)_6]^{\equiv}$ ion, a precipitate of $K_2NaCo^{III}(NO_2)_6$ results. This precipitate would presumably be present on a sprayed strip. However, there is a disadvantage in using this reagent, for the $(NH_4)_2NaCo^{III}(NO_2)_6$ precipitate is fairly soluble in water, and is partly or completely removed from a strip on washing. The sensitivity of the reaction between these alkali metals and $[Co^{III}(NO_2)_6]^{\equiv}$ ions is increased when silver salts are present, $K_2AgCo^{III}(NO_2)_6$ being formed, but this reagent cannot be employed when traces of hydrochloric acid are present in the paper.

Sodium lead cobaltous hexanitrite reagent is more sensitive than sodium cobaltic hexanitrite and has the advantage of forming a much less soluble ammonium salt. The reagent should be freshly prepared.

Detailed Procedure

1. Place the solvent-mixture, phenol - methanol - concentrated hydrochloric acid (57.5 : 22.5 : 20% W/V/V) in the solvent trough of the Shandon Chromatank and in a beaker at the bottom of the vessel, at least one hour before the experiment is started.

2. On a Whatman No. 41 filter strip, 16 cm. wide and 57 cm. long, draw pencil lines 1, 5, 6 and 7 cm. from the top end of the strip and fold the paper along the first three of these lines, so that, when the upper end dips into the solvent trough, the length from the 7 cm. mark to the end hangs vertically and gives a solvent run of 50 cm. Notch the bottom edge of the paper so that the solvent-mixture will drip off evenly when it reaches the foot.

3. Place 0.005 ml. of the test solution as a spot on the paper along the 7 cm. line. Allow the spot to dry and apply a further 0.005 ml. to the same position. Four such spots may be accommodated by placing them 4 cm. apart across the paper. Place the test solutions on a second strip in exactly the same way.

4. Allow the spots to dry and insert the strips in the chromatographic vessel. Allow the solvent-mixture to flow down the papers for 16 - 18 hours. It is very suitable to leave the papers overnight.

5. Remove the papers from the apparatus and hang them in the air for about 30 minutes; next, heat them for 10 minutes in an oven at 110°C. The papers are now ready for spraying.

6. Spray one sheet with zinc uranyl acetate reagent and hang in the air for one hour to ensure complete drying. Examine the paper under an ultra-violet lamp. Lithium and sodium fluoresce as brilliant turquoise-green spots. For examination purposes, compare the spots with a set of standards. The areas and intensities of the spots increase with increasing amounts of the elements.
7. Spray the other sheet with ethyl alcohol and immediately follow with sodium lead cobaltous hexanitrite reagent. Leave the paper in the air for 10 minutes and then wash off the pale yellow background by immersing the strip in a vessel containing tap water. Remove the strip and hang it up to dry. Compare the complex hexanitrite spots with standards as before.

Standards, Limits and Synthetic Mixtures

Using the above procedure, standards were run on Whatman No. 41 paper with 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 µM amounts of the alkali and ammonium chlorides. 0.1 µM amounts of lithium and ammonium ions were not detected although this quantity of the other metals was readily identified. The minimum amount of lithium and ammonium that could be seen was 0.25 µM. For each ion, limits

TABLE VIII

No.	Lithium	Sodium	Potassium	Rubidium	Caesium	Ammonium
1	-	0.1 (0.2)	9	0.25(0.2)	0.5	0.5
2	-	0.25(0.1)	0.5(0.1)	5 (10)	0.25(0.2)	0.5 (0.25)
3	9 (10)	0.25(0.1)	-	0.25(0.1)	0.1	0.5 (0.3)
4	2.5(3)	0.25(-)	-	0.25(0.1)	2 (7)	0.5 (0.3)
5	0.25	0.1	0.25(0.1)	-	8 (10)	-
6	-	-	0.25(0.1)	0.1	-	9 (10)
7	0.25	9	0.25(0.2)	0.25(0.2)	0.1 (0.2)	1
8	0.25	0.1	5	-	-	3 (5)
9	4 (3)	-	-	0.4 (0.1)	0.8 (0.3)	5 (7)
10	0.5	0.1(-)	0.25(0.2)	0.3 (0.2)	10	-

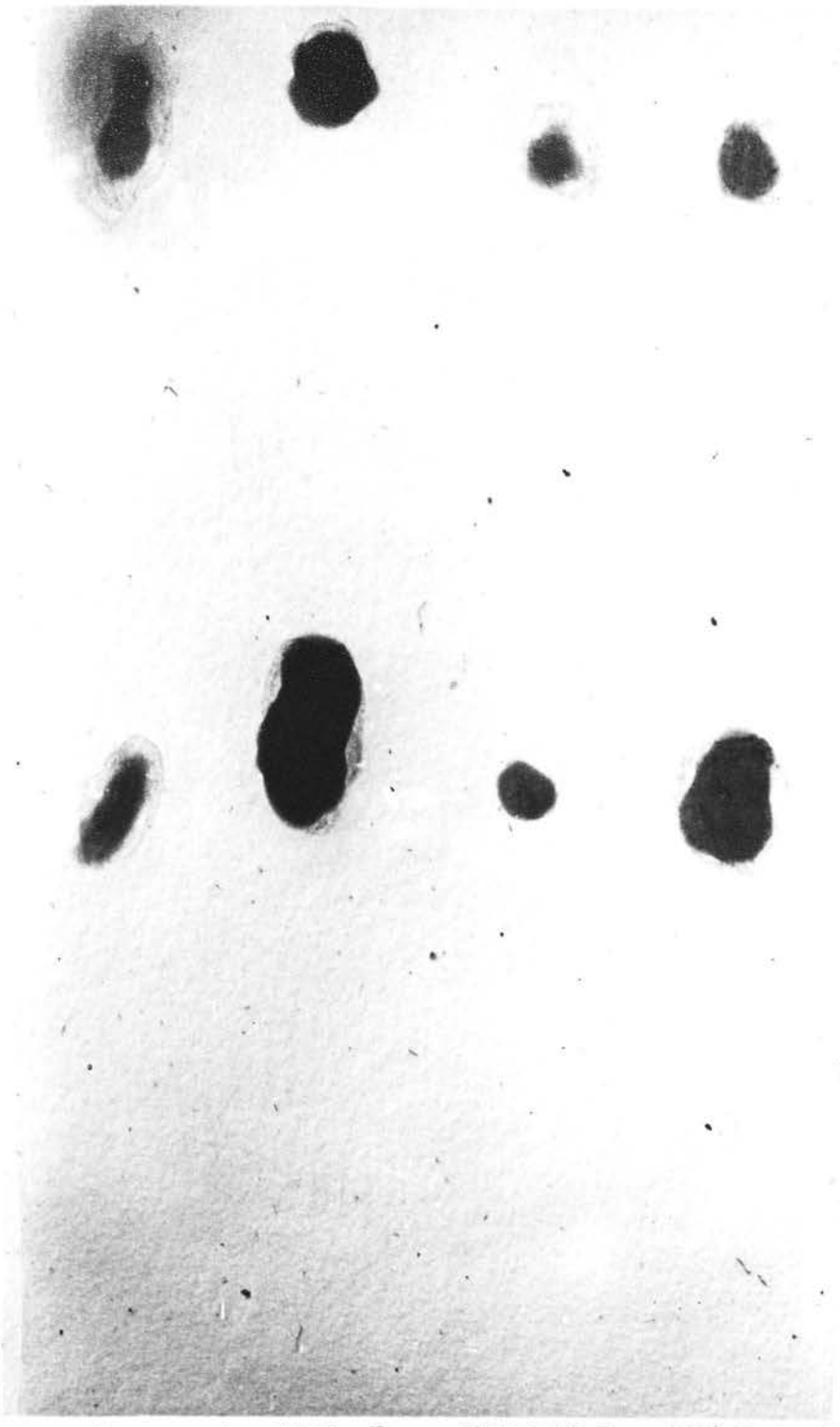
were run with 10 μM, the maximum amount being considered, associated with the minimum amounts of the other five. These were satisfactory, the minimum amounts being detected, in the presence of the maximum amount of any particular cation.

In an overnight run of 16 hr. a mixture of 1 μM of each of the six salts travelled the following distances (cm.), sodium 5.4 - 8.4, potassium 6.4 - 9.1, rubidium 10.2 - 13.3, ammonium 15.2 - 17.7, lithium 18.1 - 20.6 and caesium 21.3 - 25.3.

As a test of the validity of the proposed scheme 10 mixtures of 'unknown' composition were analysed. The results are shown in Table VIII and are the estimated amounts in micromoles of the metals found. Where these differ from the amounts present the latter are shown in parenthesis.

In nos. 4 and 10, a small amount of sodium was reported where none had been added to the mixtures. It was noticed that this sodium was present in mixtures containing large amounts of caesium and by running a chromatogram with 10 μM caesium and spraying with zinc uranyl acetate reagent, it was confirmed that the caesium stock solution did in fact contain a

PLATE 2.



low percentage of sodium as impurity. The results are considered to be satisfactory.

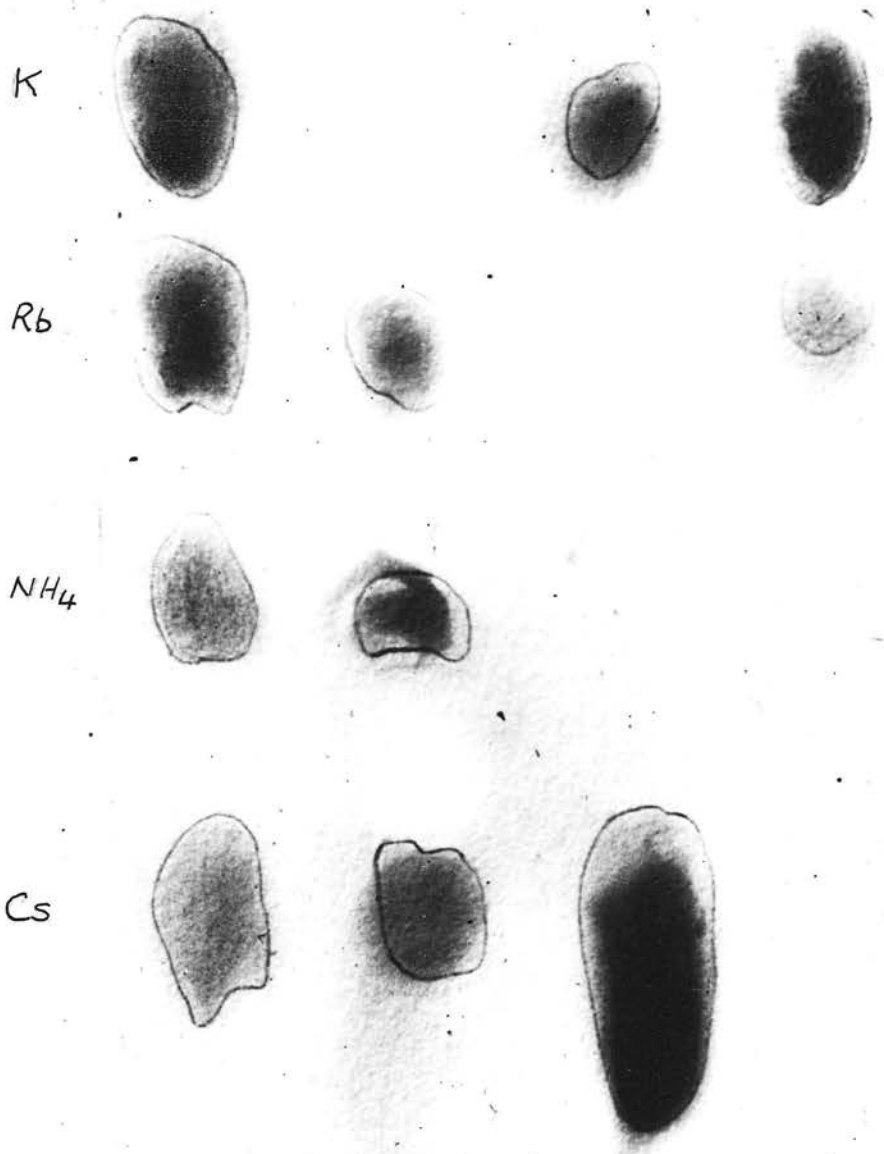
Photographs of two chromatograms are shown on Plates 2 and 3. Plate 2 is a photograph of a chromatogram sprayed with zinc uranyl acetate reagent. In order to take the photograph in daylight, the positions of the lithium and sodium spots were detected in ultra-violet light and marked with a pencil. The spots were shaded in, as skillfully as possible, to illustrate the differences in the intensities of their fluorescence. Plate 3 is a photograph of a chromatogram sprayed with sodium lead cobaltous hexanitrite reagent. The boundaries of the spots were marked with a pencil before taking the photograph. 0.01 ml. volumes of four solutions of different composition were separated on each sheet. They were (1) a mixture of 1.0 μM amounts of lithium, sodium, potassium, rubidium, caesium and ammonium, (2) mixture No. 3, (3) mixture No. 5 (see Table VIII) and (4) the solution of alkali metals from a lepidolite (see p. 66).

Application of the Method to the Analysis of Silicate Rocks

The success of the separation suggested the use of this method for the analysis of rocks containing rubidium and caesium, such as lepidolite and pollucite. The method was therefore applied to the analysis of the alkali metals present in a lepidolite. The sample of silicate rock used, had been previously analysed by Traves[37] and contained, lithium 1.6%, sodium 0.1% and potassium 5.8% (any rubidium or caesium present was of course weighed as potassium chloride). The treatment of the rock was as follows.

A Lawrence Smith fusion[38] was carried out on 50 mg. of the finely divided rock. The fused mass was digested with water for 30 min. on a steam bath and the insoluble material filtered off. The filtrate was acidified with dilute hydrochloric acid and the calcium in the solution precipitated with ammonia and ammonium carbonate solution. To remove any lithium that was present in the calcium carbonate precipitate, it was filtered off, dissolved in dilute hydrochloric acid and the calcium re-precipitated. The final solution was evaporated to dryness in a platinum

PLATE 3.



1 μM EACH MIXTURE 3 MIXTURE 5 LEPIDOLITE

Application of the Method to the Analysis of Silicate Rocks

The success of the separation suggested the use of this method for the analysis of rocks containing rubidium and caesium, such as lepidolite and pollucite. The method was therefore applied to the analysis of the alkali metals present in a lepidolite. The sample of silicate rock used, had been previously analysed by Traves[37] and contained, lithium 1.6%, sodium 0.1% and potassium 5.8% (any rubidium or caesium present was of course weighed as potassium chloride). The treatment of the rock was as follows.

A Lawrence Smith fusion[38] was carried out on 50 mg. of the finely divided rock. The fused mass was digested with water for 30 min. on a steam bath and the insoluble material filtered off. The filtrate was acidified with dilute hydrochloric acid and the calcium in the solution precipitated with ammonia and ammonium carbonate solution. To remove any lithium that was present in the calcium carbonate precipitate, it was filtered off, dissolved in dilute hydrochloric acid and the calcium re-precipitated. The final solution was evaporated to dryness in a platinum

vessel and the residue ignited to remove ammonium salts. The small residue was taken up in 0.25 ml. of dilute hydrochloric acid and 0.01 ml. portions of the solution were spotted on to the paper sheet. The separated salts wererevealed and estimated in the usual manner (see Plates 2 and 3). With the sodium estimation, an allowance was made for the trace of sodium present in the calcium carbonate used in the fusion. It was estimated on examining the chromatogram that 2 mg. of rock contained 5 μM lithium, 0.1 μM sodium, 2 μM potassium, 0.1 μM rubidium and no caesium. This corresponds to 1.7% lithium, 0.1% sodium, 3.9% potassium and 0.4% rubidium.

The use of paper chromatography for separating the alkali metals with a view to their subsequent quantitative determination using micro-techniques, is reported later.

THE APPLICATION OF PHENOL - METHANOL - CONCENTRATED HYDROCHLORIC ACID SOLVENT - MIXTURES TO THE SEPARATION OF CERTAIN OTHER ELEMENTS

The partitioning of a wide range of other metal salts between the solvent-mixture, phenol-methanol - concentrated hydrochloric acid (57.5 : 22.5 : 20% W/V/V) and the stationary phase on the paper was investigated and the R_F values for 100 µg. amounts of these metals in an overnight run on Whatman No. 1 paper are given in Table IX.

TABLE IX

Metal	R_F Value	Metal	R_F Value
Copper	0.22±0.04	Thallic	0.53±0.05
Mercuric	0.34±0.04	Manganous	0.16±0.04
Cadmium	0.30±0.05	Cobalt	0.16±0.04
Bismuth	0.20±0.04	Nickel	0.14±0.04
Antimonous	0.44±0.05	Zinc	0.35±0.05
Stannic	0.37±0.04	† Beryllium	0.23±0.10
‡ Ferric	0.39±0.05	* Magnesium	0.17±0.05
Chromic	0.12±0.04	* Calcium	0.07±0.04
Aluminium	0.10±0.05	* Strontium	0.03±0.03
Gallium	0.66±0.06	* Barium	0
Indium	0.20±0.05		

† sulphate * nitrate The others were chlorides.

‡ Iron gave another faint spot at 0.18±0.03. This is probably due to Fe^{+++} and the other to $FeCl_4^-$.

It can be seen that, in addition to the Group IA metals, this solvent-mixture will separate many elements including those of Group IIIB of the Periodic Table, viz. aluminium, gallium, indium and thallium. The separation of these latter elements with aliphatic alcohols saturated with aqueous hydrochloric acid solution has been mentioned before[39], [40] but no previous investigators had used solvent-mixtures containing phenols. Improvements in the separation of the Group IIIB metals were made on Whatman No. 1 paper, by varying the ratios of phenol, methanol and acid. They were separated with solvent-mixtures containing 20 - 40% of concentrated hydrochloric acid and 15 - 20% of methanol. The best separation was given with phenol - concentrated hydrochloric acid - methanol (50 : 30 : 20% W/V/V). The positioning of iron, zinc, titanium and zirconium was also investigated with this solvent-mixture.

Procedure

The papers are prepared and the chromatograms run in a similar manner to that recommended for the alkali metals.

Detection of Aluminium, Gallium, Indium, Zinc and Iron: Spray the paper with a 5% W/V solution of 8- hydroxyquinoline in methanol - chloroform - water (85 : 10 : 5% V/V) and hang it in a closed vessel containing a beaker of concentrated ammonia solution. Iron appears as a black spot. Allow the paper to dry and examine under an ultra-violet lamp. Aluminium, gallium, indium and zinc fluoresce brilliantly as yellow spots.

Detection of Thallium, Titanium and Zirconium:

The spraying reagents are as follows.

Thallium - A 5% W/V aqueous solution of potassium iodide

Titanium - A 1% W/V aqueous solution of chromotropic acid

Zirconium - Ethanol saturated with alizarin.

Spray the paper with the appropriate reagent. Thallium is shown as a yellow spot, titanium as red-brown and zirconium appears as purple.

Results

The R_F values of 100 µg. amounts of these metals run with phenol - concentrated hydrochloric acid - methanol (50 : 30 : 20% W/V/V) in an overnight run (16 hr.) are given in Table X.

TABLE X

Element	R_F Value	Element	R_F Value
Zirconium	0.09±0.06	Zinc	0.40±0.05
Aluminium	0.13±0.05	Thallic	0.58±0.06
Indium	0.28±0.04	Iron	0.64±0.06
Titanium	0.34±0.06	Gallium	0.73±0.07

1 µg. amounts of aluminium, gallium, indium and zinc are readily detected with 8-hydroxyquinoline and 1 µg. gallium can be separated from 1000 µg. aluminium with the solvent—mixture.

These results suggest that the solvent-mixture could be used for the qualitative analysis of certain aluminium alloys. As a test of this procedure, 500 µg. amounts of No. 181 Aluminium Alloy 'A' (supplied by British Chemical Standards) containing

Cu	Zn	Ni	Pb	Mg	Fe
23 µg.	12 µg.	9 µg.	7 µg.	6 µg.	3 µg.

were separated on Whatman No. 1 paper. The individual substances were revealed with the following spraying reagents, viz. iron, aluminium, magnesium and zinc - 8-hydroxy-quinoline, lead - sodium rhodizonate, and copper and nickel - rubeanic acid. All these minor constituents were detected.

INVESTIGATIONS ON THE QUANTITATIVE DETERMINATION OF POTASSIUM, RUBIDIUM AND CAESIUM AFTER THEIR SEPARATION BY PAPER CHROMATOGRAPHY

A mixture of the alkali metals may be separated completely into lithium, sodium and potassium - rubidium - caesium, by the classical methods, but no method based on precipitation exists for the complete separation of the elements of this last group.

A mixture of these elements may however be analysed without a separation, if a flame photometer is employed. Modern flame photometers are developments of an instrument described in 1945 by Barnes and co-workers[41]. With that instrument the interferences due to foreign matter present in the solution were studied. While good results were obtained for pure solutions, it was found that the presence of other substances in the solution affected the true result. Berry and co-workers[42] described an instrument employing an 'internal standard' to cut down on the interferences due to foreign material. While such interferences are seldom removed completely, they are usually reduced by this method. However, lithium is generally used as an 'internal standard' and such an instrument

could not be used for alkali mixtures containing that element.

In addition to these interference effects, the flame photometric analysis of mixtures containing potassium and rubidium presents a special problem. The principal emission lines of potassium and rubidium are the doublet at 766.5 and 769.9 mμ, and 780.0 mμ, respectively. No instrument employing filters could resolve these bands, since combinations of filters will allow through a band of 30 - 40 mμ width at the best. Potassium and rubidium, when together, could only be determined with an instrument using a band width not exceeding 20 mμ. A determination of these two elements should be possible on a modern flame photometer such as the Unicam SP 900.

However, it is doubtful if results of the highest accuracy could be obtained on a flame photometer and indeed, Parks and co-workers[43] have recommended the removal of interfering ions from complex mixtures of unknown composition as the only means of obtaining accurate results. It was therefore decided to investigate the possibilities of determining quantitatively the microgram

amounts of potassium, rubidium, caesium and ammonium that had been separated with phenol-methanol - concentrated hydrochloric acid (57.5 : 22.5 : 20% W/V/V) on Whatman No. 41 paper.

A consideration of the possibilities indicated that there were two ways by which the separated cations might be quantitatively determined. In the first method, a strip containing the separated salts could be sprayed with sodium lead cobaltous hexanitrite reagent. The potassium, rubidium, caesium and ammonium ions would be precipitated as their complex hexanitrites. According to Cuttica[44], the formulae of the precipitates correspond to M_2 Pb Co ^{II} $(NO_2)_6$ where M is the alkali metal. Sergienko[36] has also used sodium lead cobaltous hexanitrite reagent for the quantitative determination of potassium. The excess spraying reagent could be removed by washing, and the amount of each alkali determined from the cobalt content of each hexanitrite precipitate.

In the second method, two equal volumes of the alkali solution are spotted on to the same sheet of filter paper and the chromatogram

developed. The paper is cut longditudinally in half and only one of the sets of separated salts is sprayed to reveal the positions of the cations. This half-strip may be referred to as the control. By placing the control alongside the unsprayed half of the sheet and, by extending to this sheet, pencil lines drawn to mark out the areas of paper containing the hexanitrite precipitates, it is possible to obtain on the unsprayed strip sections of paper each containing a microgram quantity of either potassium, rubidium, caesium or ammonium ion. From each of these sections of paper the alkali metal chlorides may be removed with water or dilute acid and determined quantitatively by micro-analytical methods.

The first of these suggested methods was investigated. Rubber gloves were worn when it was necessary to handle filter-paper sheets prior to placing them in the chromatographic vessel. For the method to be successful it was necessary that all the excess spraying reagent should be removed on washing with water but this washing could not be continued for more than a few minutes

as there would be a risk that part of the precipitates might dissolve in the water.

2.36 μM potassium, 2.06 μM rubidium, 2.36 μM caesium and 2.06 μM ammonium chloride were separated on a chromatogram and revealed with the spraying reagent. Excess reagent was washed from the paper, the precipitates dissolved from the paper and the cobalt content of each solution found as outlined below.

Cobalt Estimations

The following procedure was based on the method of Haywood and Wood[45] for the photometric determination of cobalt in alloy steels.

Reagents: Sodium Acetate Solution - 500 ml. containing 250 g. of "Analar" solid
Nitroso R salt solution - 1 g. of solid (supplied by Hopkin and Williams, Ltd.) in 500 ml. of water.
Standard Cobalt Solution containing 1 μM Co /ml. i.e. 98.8 mg. of cobalt ammonium sulphate (B.D.H. low in nickel and iron) in 250 ml. solution.

Cobalt in Complex Hexanitrites and Blank: The salt was removed quantitatively from the paper with 2 ml. of N nitric acid followed by 10 ml. of 0.1 N nitric acid and the paper washed

with a further 10 ml. of water.* 10 ml. of nitroso R salt solution and 10 ml. of sodium acetate solution were added and the solution heated to boiling. 5 ml. of concentrated nitric acid were added, the solution boiled for not less than one minute and not more than two minutes, then cooled and made up exactly to 100 ml. with water.

Calibration Curve: Six 100 ml. conical flasks were used. 5, 4, 3, 2 and 1 ml. portions of the standard cobalt solution were added to flasks 1 to 5 respectively, a burette being used. 3 ml. of N nitric acid and 10 ml. of water were added to each flask. 10 ml. of nitroso R salt solution were added to each and the volumes made approximately the same by adding water. To each flask, 10 ml. of sodium acetate solution were added and the solutions further treated as above.

On the spectrophotometer, set at 470 mμ[46], the absorptions of the potassium, rubidium, caesium, ammonium and blank solutions, and solutions 1 to 5, were measured against solution 6 (which contained nitroso R salt but no cobalt). Optical density was plotted against concentrations for solutions 1 to 5 and from the straight line graph, the cobalt content

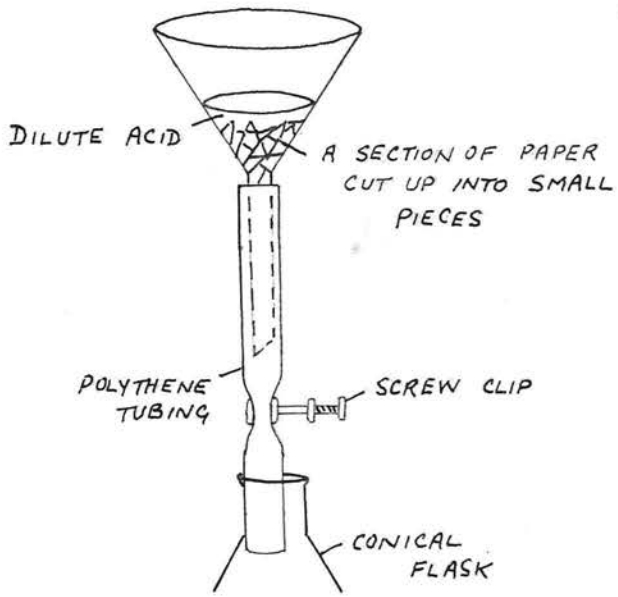

DILUTE ACID
A SECTION OF PAPER CUT UP INTO SMALL PIECES
POLYTHENE TUBING
SCREW CLIP
CONICAL FLASK

FIGURE 3.

of the other solutions was determined.

* The method used for removing the precipitate from the paper was very simple. The stems of 1½" filter funnels were pushed into 4" lengths of polythene tubing (5 mm. internal diameter). A section of paper containing one of the separated alkali salts was cut up into small pieces which were placed in a funnel. The polythene tube was closed by a screw clip and dilute acid added to the funnel until the paper was completely covered with liquid. After a few minutes the liquid was run into a flask by opening the screw clip. This operation was repeated one or more times if necessary. The filter paper was then thoroughly washed with water. A sketch of the apparatus is shown in Figure 3.

Results

The amounts of cobalt in those parts of the paper containing the precipitates were 2.10 μM for potassium, 1.76 μM for rubidium, 1.40 μM for caesium and 1.94 μM for ammonium lead cobaltous hexanitrite. The paper blank contained approximately 0.03 μM of cobalt per sq. cm. As the blank would almost certainly vary over the whole area of the paper, the percentage recoveries for the

alkali metals could not be calculated with any degree of accuracy. The most soluble of the complex hexanitrites was known to be that of caesium and the results seem to bear this out. 1.40 μM of cobalt were recovered from that part of the paper containing the caesium precipitate and if 0.80 μM of this is estimated for the excess reagent not removed from the paper on washing, this leaves about 0.60 μM cobalt for the actual caesium lead cobaltous hexanitrite precipitate. If the formula for the precipitate is $Cs_2\ PbCo^{II}(NO_2)_6$, the recovery was about 50%. It would therefore be useless to try and lower the cobalt content of the blank by continuing the washing for already 50% of the caesium precipitate has been removed on 2 to 3 minutes washing. The method was therefore abandoned.

It now appeared that accurate results would only be obtained by using the second method outlined above i.e. by removing the separated salts from the paper with water or dilute acid and determining the alkali metal content of the resulting solutions. Duval and Doan[46] have investigated a number of methods that can be applied to the quantitative analysis of microgram amounts of caesium which have

been separated from potassium and rubidium with Miller and Magee's solvent-mixture[4]. They preferred a method where the caesium was precipitated as caesium cobaltic hexanitrite and the nitrite in the precipitate determined colorimetrically with sulphanilic acid, 1-naphthylamine and sodium hydroxide. Unfortunately, however, potassium and rubidium do not form precipitates of a definite composition with sodium cobaltic hexanitrite reagent.

No investigations were made on the quantitative determination of potassium, rubidium and caesium by the second method but it is suggested that the best results would be obtained by precipitating these alkali metals from the solutions with sodium tetraphenyl boron reagent and determining the amounts of precipitate formed by a titrimetric method. Flaschka and his co-workers[47], [48] have outlined two methods for the quantitative determination of microgram amounts of potassium with this reagent. There seems to be no reason why rubidium and caesium could not be determined in a similar manner.

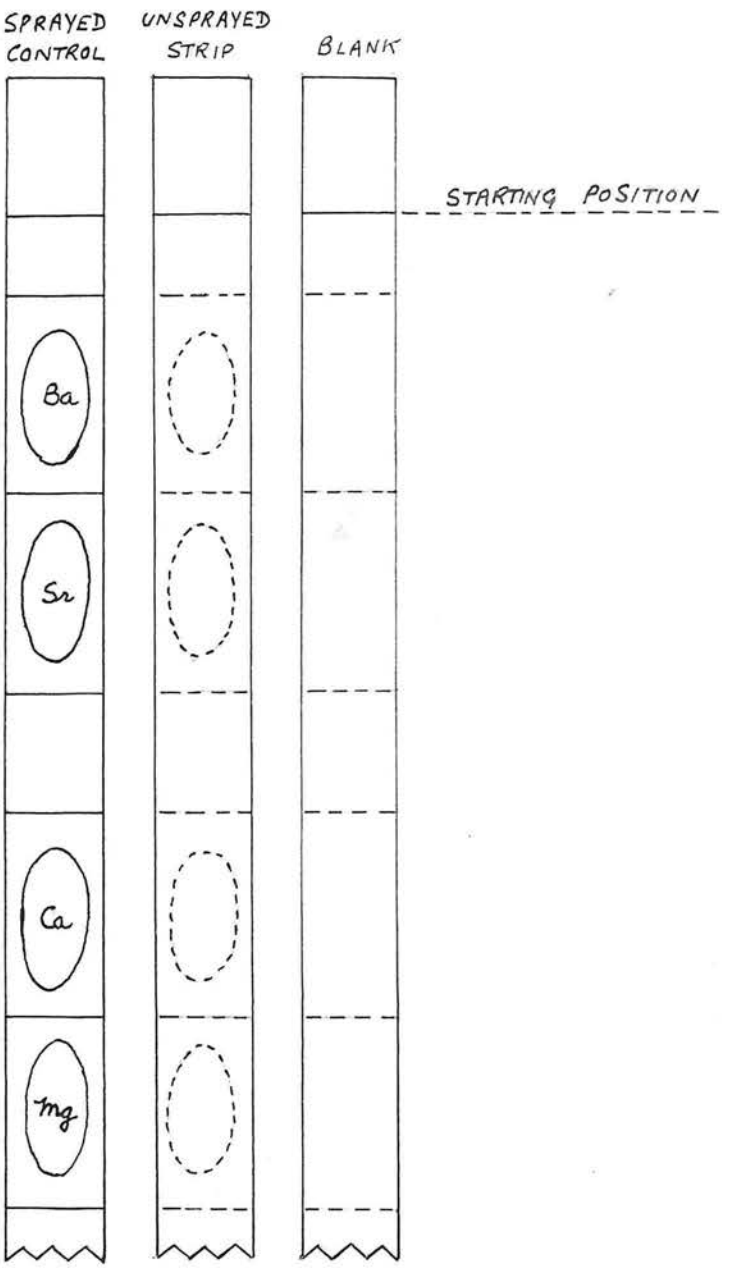

SPRAYED CONTROL
UNSPRAYED STRIP
BLANK
STARTING POSITION
Ba
Sr
Ca
Mg

FIGURE 4.

THE QUANTITATIVE DETERMINATION OF MAGNESIUM AND THE ALKALINE EARTHS AFTER THEIR SEPARATION BY PAPER CHROMATOGRAPHY

Following the success of the solvent-mixture, methanol - n-butanol - symm. collidine - 6N acetic acid (40 : 20 : 20 : 20% V/V) for the separation of 0.1 - 10.0 µM amounts of magnesium and the alkaline earths, it was decided to investigate methods for the quantitative determination of the separated cations.

Removal of the Separated Cations from the Papers

Three ways of removing the separated salts from a paper were investigated. These were, (1) dissolving the material from the separated spots on a sprayed strip with concentrated acid, (2) cutting out the separated spots from a sprayed strip and destroying the organic material by a wet or dry ashing process, and (3) separating two identical amounts of the mixture on the same sheet of paper, spraying only the control to find the positions of the spots (c.f. alkali metals p. 75) and from the unsprayed strip dissolving out the separated salts with water or dilute acid. This is illustrated in Figure 4.

The first method was unsatisfactory, since the spraying reagents, which were extracted from the paper, interfered with the

subsequent determinations. It was possible to use both the second and third procedures, but the third was preferred, since less foreign inorganic material would be present in the resulting solution. The required salt was extracted from the paper in a filter funnel as described under the section on the alkali metals (p. 78).

Because of the relatively high calcium content of Whatman No. 3MM paper, the double acid washed Whatman No. 41 paper was used for quantitative work. On this paper, a total of 10 µM of the cations can be separated but with certain restrictions, namely that the amounts of the pairs magnesium - calcium and strontium - barium should not exceed 5 µM.

Preliminary Investigations on the Quantitative Determination of these Elements

Various methods were considered for determining the cations after their removal from the paper.

Magnesium: A modification of the colorimetric method of Gillam[49] based on the red lake formation with Titan Yellow on the hydroxide was investigated and seemed possible.

Calcium: A modification of the method of Lindner and Kirk[50] in which calcium is first precipitated as oxalate, dissolved in dilute sulphuric acid, ceric sulphate solution added and the excess oxidising reagent back-titrated with ferrous ammonium sulphate using o - phenanthroline ferrous sulphate as indicator, was investigated and was quite satisfactory.

Strontium and Barium: The possibility of precipitating these two elements as carbonates, dissolving the precipitates in hydrochloric acid solution and back-titrating with sodium hydroxide solution using bromo-phenol blue as indicator was examined. The method was unsatisfactory since the results were consistently low.

Although as stated, it would be possible to use the methods outlined above for magnesium and calcium, it was considered more convenient to search for an analytical method that could be used for all these cations. Titrations of the solutions with ethylene diamine tetra-acetate were therefore investigated.

SPECTROPHOTOMETRIC TITRATIONS WITH DI-SODIUM ETHYLENE DIAMINE TETRA - ACETATE.

Magnesium and the alkaline earths form stable water-soluble complexes with ethylene

diamine tetra-acetic acid. The stability constants of these complexes have been found[51]; the pK values are magnesium 8.7, calcium 10.6, strontium 8.6 and barium 7.8, where

$K = \frac{[M^{++}][X^{==}]}{[MX^{=}]}$ (M^{++} being the metal ion and $X^{==}$ the ethylenediamine tetra-acetate ion). Biedermann and Schwarzenbach[52] suggested that magnesium should be titrated with ethylene diamine tetra-acetate in a solution buffered at about pH 10 with ammonia and ammonium chloride, using solochrome black as indicator. The colour change is from wine-red to blue. Calcium, strontium and barium do not form stable complexes with the indicator. Murexide forms a stable complex with calcium[53], [54] and can be used to detect the end-point when a solution of that metal is titrated at pH 12. Any magnesium in the solution is precipitated as the hydroxide at that pH and is not titrated. Strontium and barium form only weak complexes with murexide. Calcium, strontium and barium are satisfactorily determined in a titration with di-sodium ethylene diamine tetra-acetate, when solochrome black is used as indicator, if a known quantity of magnesium is added to each solution before the titration is started[52], [55], [56], [57]. The solution is buffered to

pH 10 with ammonia - ammonium chloride solution.

In visual titrations, there is a limit, below which the indicator concentration cannot fall, if the end-point is to be readily detected. When this minimum amount of indicator is added to microgram quantities of magnesium, a fair percentage of the magnesium is still complexed to the indicator and a sharp end-point is not obtained when the solution is titrated with ethylene diamine tetra-acetate. For this reason, a spectrophotometric method was adopted for the determination of the salts that had been removed from the chromatographic papers. A spectrophotometric titration was first used by Sweetser and Bricker[58] for the quantitative determination of iron, cobalt and nickel with ethylene diamine tetra-acetate.

Between pH values 6.3 and 11.5 solochrome black is blue but the magnesium complex is wine-red. By plotting a graph of optical density as a function of wave length for the free dye and the complex, Karsten and co-workers[59] found that the greatest differences in absorption occurred at 530 and 630 mμ and recommended that these wave lengths be used for titrations of magnesium ion with ethylene

Titrations of Magnesium Ion at 530 mμ and 630 mμ.

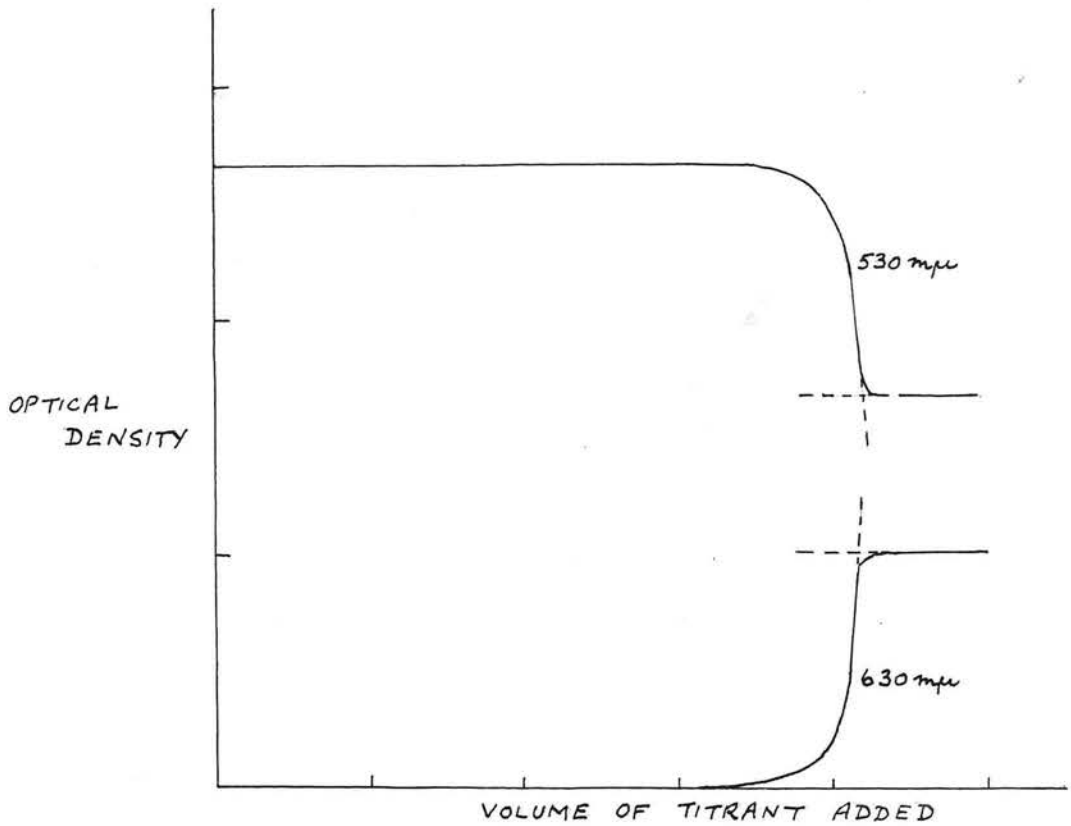


FIGURE 5.

diamine tetra-acetate solution when solochrome black is used as indicator. If titrations are made at 630 mμ the light absorption increases, i.e. the optical density readings rise, when the end-point is approached until a constant value is obtained for the pure blue solution. At 530 mμ, a decrease in optical density occurs near the end-point. These titration curves are shown in Figure 5. The true end-point has been reached when the constant value is attained. When the titration conditions are favourable a sharp break in the graph of optical density against volume of titrant added, occurs at the end-point.

The theory of the spectrophotometric titration was worked out by Fortuin and co-workers[60]. Perfectly sharp end-points are obtained if

(1) ${}^{*}K_c \gg {}^{*}K_I$ (numerically speaking, $\log \frac{{}^{*}K_c}{{}^{*}K_I}$ should be at least 4)

(2) ${}^{*}K_I$ is large (numerically speaking, $10^4 - 10^5$)

(3) i_t is low

(4) m_t is as high as possible,

where ${}^{*}K_c$ = complex-forming capacity of the titre substance.

${}^{*}K_I$ = complex-forming capacity of

the indicator.

i_t = total concentration of the indicator

m_t = total metal ion concentration.

The first of these criteria is the most important. The end-point becomes less sharp as the value of $\log \frac{^*K_c}{^*K_I}$ decreases. However, satisfactory end-points may still be obtained provided that this value exceeds 3.

For magnesium ions and solochrome black indicator at pH 9 - 10, a value for $^*K_I$ of $10^{4.5 - 5.5}$ has been given[59]. In a solution buffered at pH 10 the complex-forming capacities ($^*K_c$) of ethylene diamine tetra-acetate with magnesium and the alkaline earths are magnesium 1.7×10^8, calcium 1.4×10^{10}, strontium 1.5×10^8 and barium 2.1×10^7. A study of these values indicated that the titration curves would be reasonable for magnesium, calcium and strontium but less satisfactory for barium. This was confirmed by later work.

Spectrophotometric titrations have also been used by Chalmers[61], and Hunter and Miller[62] for the determination of small amounts of calcium and zinc respectively.

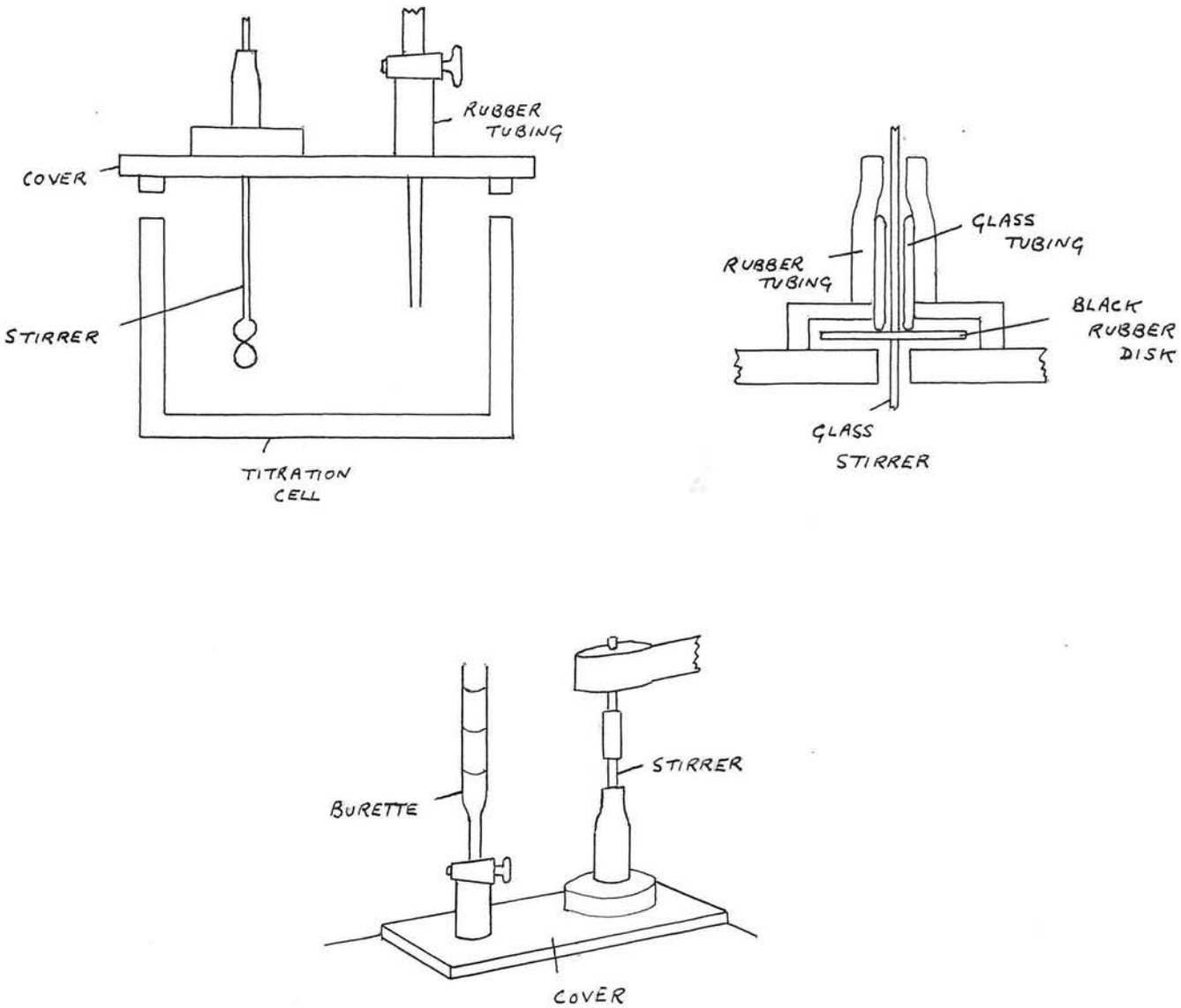

RUBBER
TUBING
COVER
STIRRER
TITRATION
CELL
GLASS
TUBING
RUBBER
TUBING
BLACK
RUBBER
DISK
GLASS
STIRRER
STIRRER
BURETTE
COVER

FIGURE 6.

Apparatus

The arrangement of the apparatus for the spectrophotometric titrations on the Unicam SP 500, was similar to that used by Hunter and Miller for the titration of zinc solutions[62] and is shown in Figure 6. 100 ml. of solution were titrated in a vessel which completely filled the cell compartment of the spectrophotometer. This cell was constructed from 3 mm. 'Perspex' Sheet and had external dimensions 9.9 x 4.7 x 6.2 cm. The cell compartment was closed by a 'Perspex' cover which had two openings in it for a 5 ml. burette and a microstirrer. The stirrer (supplied by Voss Instruments Ltd.) was suitably driven by a 2 volt accumulator in series with a variable resistance. Opaque paper was stuck to the cover with chloroform to make it light-proof and precautions were taken to exclude as much light as possible from the cell compartment.

Since 'Perspex' is opaque to ultra-violet light, a special cell with quartz windows was constructed for use in the wave length region of 200 - 400 mμ. This cell had the same dimensions as the ordinary 'Perspex' cell but contained two quartz windows of dimensions, 3.4 x

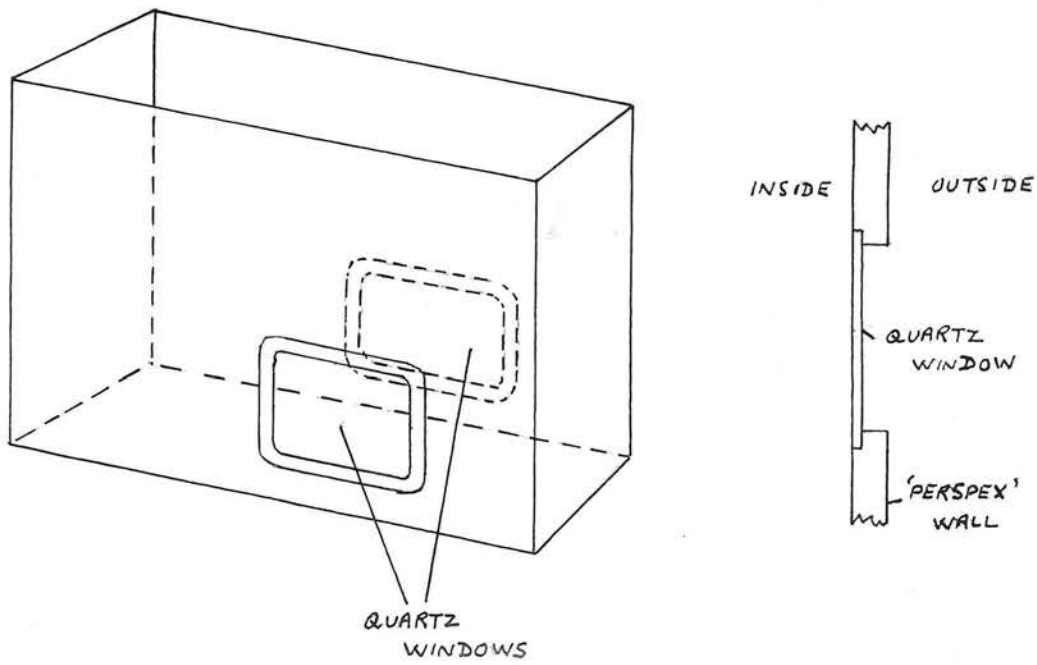


FIGURE 7.

2.5 x 0.1 cm. These were constructed from a 3" x 1" fused silica microscope slide of 1 mm. thickness (supplied by Thermal Syndicate Ltd.) and were sealed to the 'Perspex' with Araldite 101 cold-setting resin mixed with Hardener 951 (supplied by Aero Research Ltd.). The windows were inserted in such a way that the monochromatic light beam passed through the centre of them, to the photo-electric cell. A sketch of the special 'Perspex' cell is shown in Figure 7.

Reagents

These were all of "Analar" grade where possible.

Di-sodium di-hydrogen ethylene diamine tetra-acetate:- An approximately 0.0025 M aqueous solution.

Magnesium, calcium, strontium and barium chloride solutions:- Exactly molar. Other standards were made from these by dilution.

Ammonia - Ammonium Chloride Solution A:-

6.75 g. ammonium chloride and 57 ml. concentrated ammonia solution in 100 ml. solution.

Ammonia - Ammonium Chloride Solution B:-

5.40 g. ammonium chloride and 57 ml. concentrated ammonia solution in 100 ml. solution.

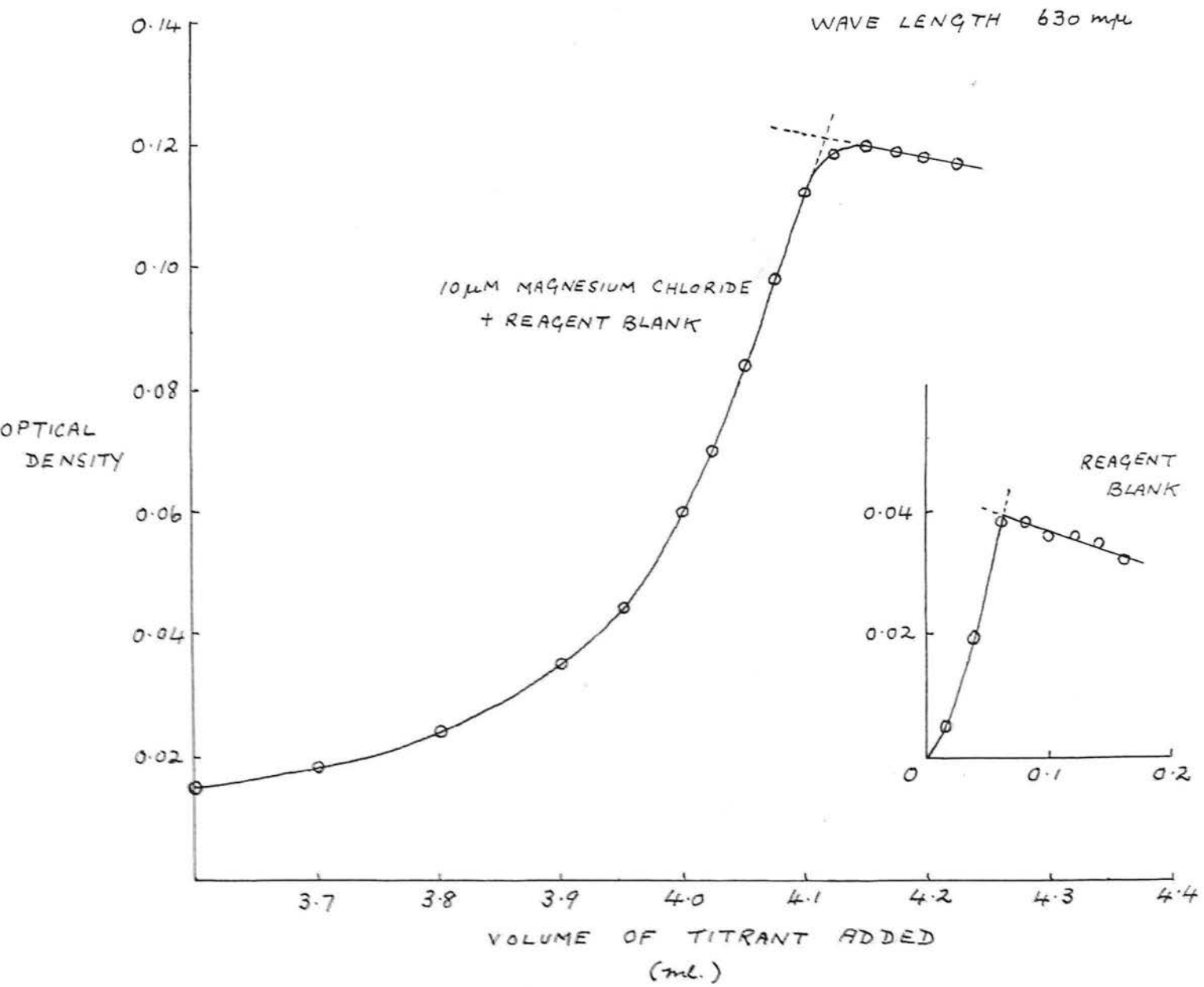

WAVE LENGTH 630 mμ
0·14
0·12
0·10
0·08
0·06
0·04
0·02
OPTICAL DENSITY
10 μM MAGNESIUM CHLORIDE
+ REAGENT BLANK
REAGENT BLANK
0·04
0·02
0
0·1
0·2
3·7
3·8
3·9
4·0
4·1
4·2
4·3
4·4
VOLUME OF TITRANT ADDED
(ml.)

FIGURE 8.

Ammonia Solution C:- 57 ml. concentrated ammonia solution in 100 ml. solution.

Indicator Solution:- A centrifuged solution of 50 mg. of solochrome black and 450 mg. of hydroxylamine hydrochloride in 10 ml. of anhydrous methyl alcohol. This was the solution recommended by Heald and co-workers[63] except that anhydrous methyl alcohol was substituted for ethyl alcohol.

Ascorbic acid:- A fresh 1% W/V aqueous solution.

Potassium Cyanide:- A 0.4% W/V aqueous solution.

Ammonium Tartrate:- A 0.2% W/V aqueous solution.

Titrations in Simple Buffered Solutions.

Standardisation of di-sodium ethylene diamine tetra-acetate solution:

An approximately 0.0025 M solution was standardised at 630 mμ in titrations with 10 μM amounts of magnesium chloride contained in 100 ml. of solution buffered at pH 10 with ammonia - ammonium chloride solution A and containing 0.1 ml. of solochrome black indicator solution. Corrections were made for the indicator blank. The titres were concordant to 3 parts in 1000. Typical titration curves for 10 μM of magnesium chloride and the blank are shown in Figure 8. Since there is a slight rounding of the graph at the end-point, the true

end-point is obtained by extending the straight parts of the graph until they intersect. There was a fading of the indicator during these titrations and this was troublesome if the titrations were not completed within 15 minutes.

Extraction and Titration of Magnesium, Calcium, Strontium and Barium from Chromatographic Papers:

A mixture of 2.5 μM amounts of each of these chlorides was separated on a chromatogram and each cation extracted from the paper with 10 ml. of 0.05 N hydrochloric acid solution followed by 10 ml. of water. 0.5 μM of magnesium chloride were added to each solution together with 2 ml. of the ammonia - ammonium chloride solution B and 0.1 ml. of the indicator solution. The solutions were titrated with ethylene diamine tetra-acetate solution and the end-points found from the optical density - volume curves. The chromatographic paper blanks were treated in a similar way. The recoveries (corrected for the blanks) for each cation are shown in Table XI.

TABLE XI/

TABLE XI

Cation	Amount in the mixture (μM)	Amount Recovered (μM)
Magnesium	2.50	2.49
Calcium	2.50	2.43
Strontium	2.50	2.38
Barium	2.50	2.25

The results, especially for barium, indicated that 0.05N hydrochloric acid did not remove all of the cations from the paper. In later work N hydrochloric acid was employed.

Titrations in the Ultra-Violet Range

Fading of the indicator was troublesome in the above titrations, so attention was turned to the possibility of a titration of magnesium, etc. with ethylene diamine tetra-acetate at pH 10 in the ultra-violet region. Sweetser and Bricker[64] have stated that the $X^{==}$ and $MX^{=}$ ions have different absorption curves in the short ultra-violet part of the spectrum (H_4X represents ethylene diamine tetra-acetic acid and M^{++} a divalent cation). For magnesium and calcium the greatest difference in absorption occurs at 222 mμ[64]. Using 1 cm. quartz cells on the Unicam SP 500 spectrophotometer and solutions of 2.4×10^{-3}M ethylene

The effect of the addition of ~0.0025 M di-sodium ethylene diamine tetra-acetate solution to (1) 100 ml. of a solution buffered at pH10 and containing 50 μM of magnesium chloride, and (2) 100 ml. of a solution buffered at pH10 and containing no titratible cations.

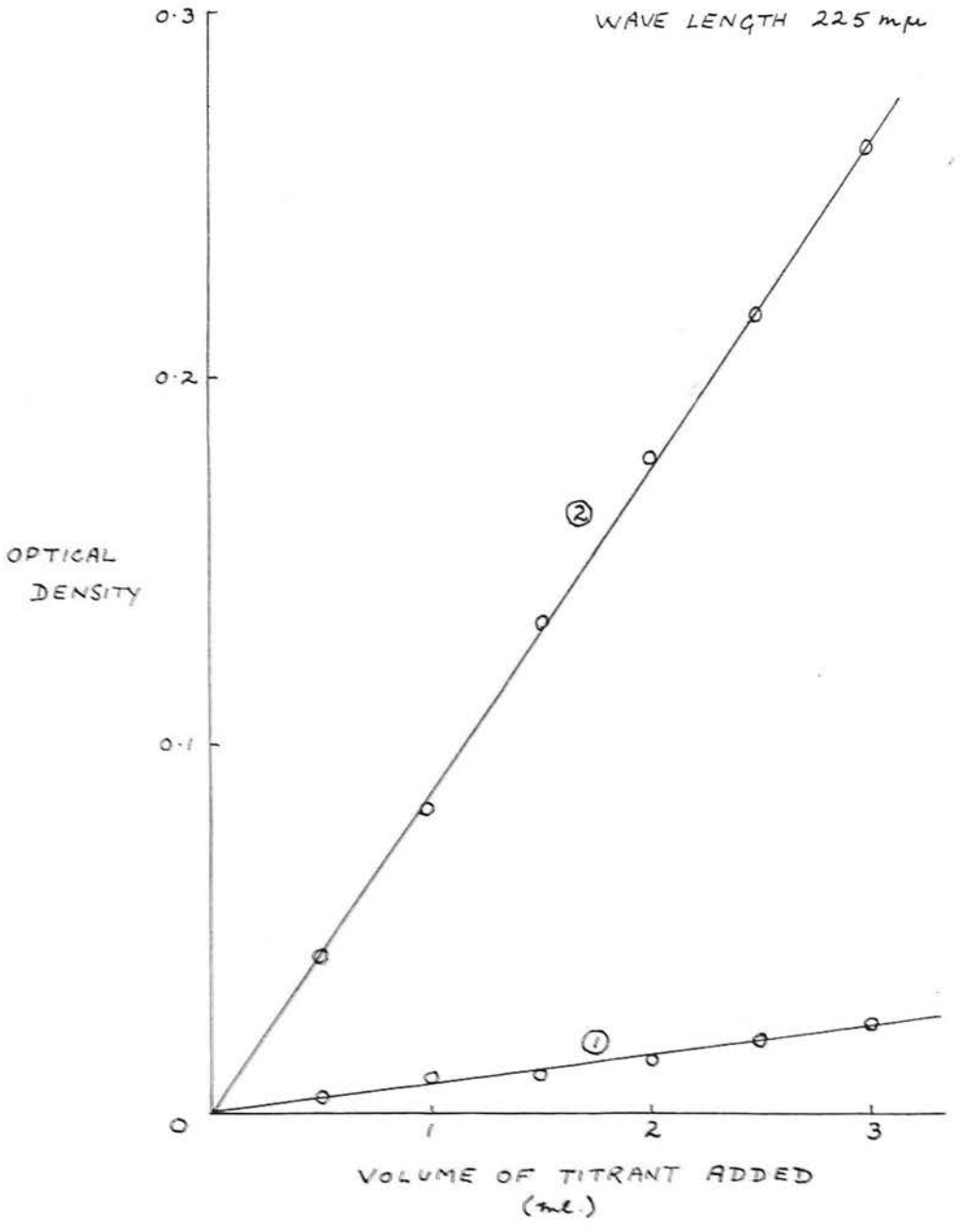


FIGURE 9.

diamine tetra-acetate, graphs of optical density as a function of wave length were drawn for solutions of the $X^{==}$, $MgX^{=}$, $CaX^{=}$, $SrX^{=}$ and $BaX^{=}$ ions. The curves for the last four ions were very similar. A plot of the difference between the optical density readings for $X^{==}$ and $MX^{=}$ ions gave a maximum at 218 mμ, a value which agrees well with that of Sweetser and Bricker(64).

When the 'Perspex' cell with quartz windows (p. 88) was filled with water and placed in the cell compartment of the spectrophotometer, the instrument could not be balanced at wave lengths < 220 mμ and titrations made in this cell were carried out at 225 mμ. To get some information about the optical density rises during a titration, a solution of approximately 0.0025 M ethylene diamine tetra-acetate was added in 0.5 ml. increments to (1) 100 ml. of solution at pH 10 containing 50 μM magnesium chloride and (2) 100 ml. of water buffered at pH 10 with ammonia and ammonium chloride. The curves for the $MX^{=}$ and $X^{==}$ ions are shown in Figure 9. They are linear. These curves indicated that an ethylene diamine tetra-acetate solution of concentration approximately 0.0025 M would be satisfactory for titrating amounts of magnesium, etc. from 0.1 - 10.0 μM.

The titration of 5 μM of magnesium chloride with ~0.0025 M di-sodium ethylene diamine tetra-acetate solution.

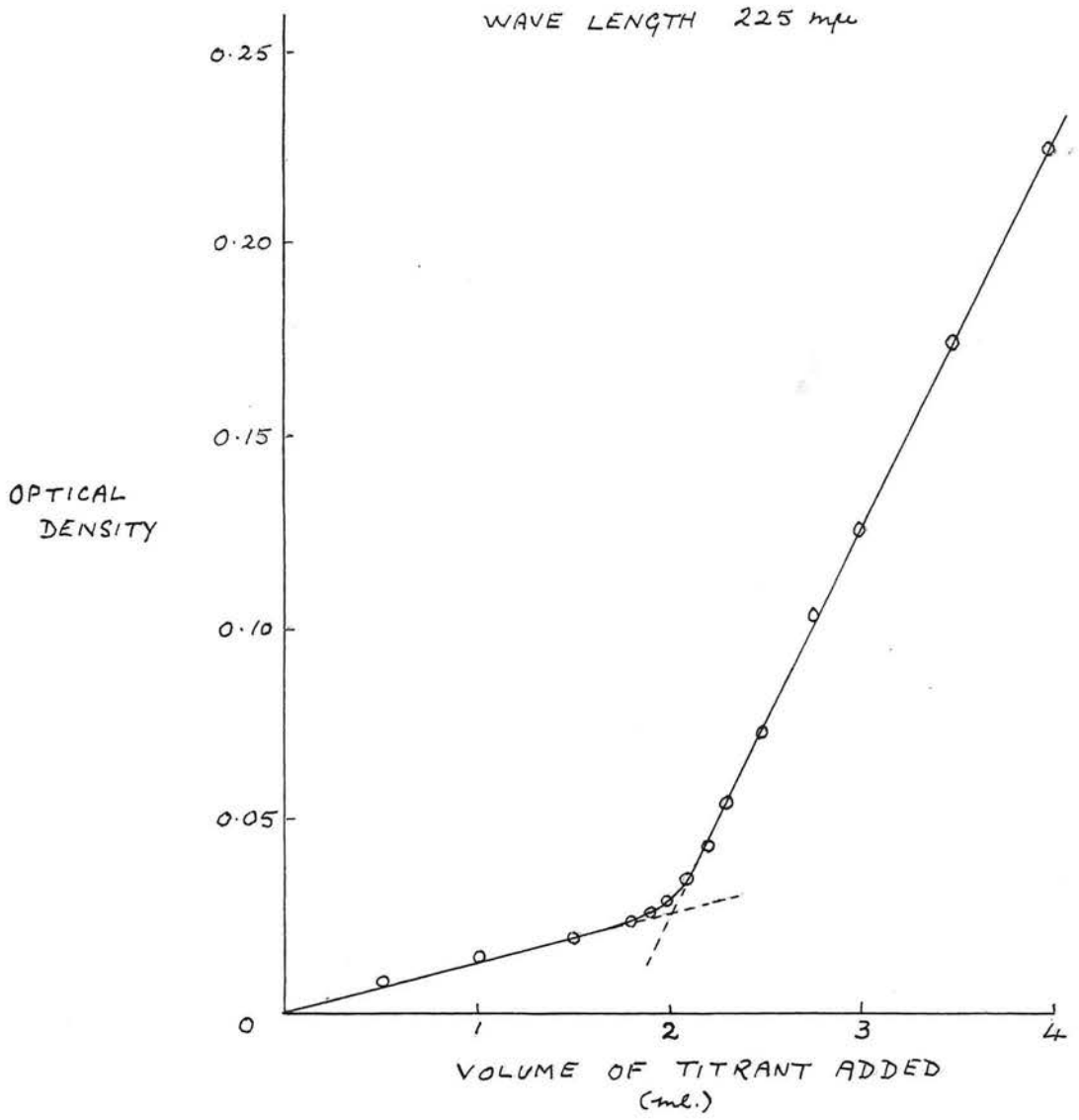


FIGURE 10.

Using an ethylene diamine tetra-acetate solution previously standardised at 630 mμ, 5 μM of magnesium chloride were titrated at 225 mμ. The curve was satisfactory and is reproduced in Figure 10. The end-point occurred at 2.03 ml. compared with 2.07 ml. for the titration of a similar quantity at 630 mμ (no blank corrections being made in either case). 4 μM barium chloride, titrated at 225 mμ, gave a similar graph to that obtained for magnesium.

However, the titrations at 225 mμ gave less concordant results than those at 630 mμ. This is due to two reasons. Firstly, to plot the curve at 225 mμ, the abscissa must cover a range of about 4 ml. but for titrations at 630 mμ, a range of only 0.5 ml. is required i.e. at 225 mμ the error of plotting is much greater. Secondly, a very wide slit (1.5 - 2.0 mm.) is required at 225 mμ and this results in a slight unsteadiness in the galvanometer readings. It would also be difficult to get accurate results for the blanks, if the titrations were done in the ultra-violet region and so the method of titration at 630 mμ was preferred.

Titrations of Solutions treated with Cyanide

The main drawback to the titrations at 630 mμ, where solochrome black was used, was the fading of the indicator that occurred during the titration. This is attributed to a reaction between traces of iron and the indicator. Hunter and Miller[62] were able to eliminate interferences from iron, copper and certain other elements in titrations of zinc with ethylene diamine tetra-acetate by first reducing the iron to the ferrous state with ascorbic acid in a slightly acid solution and complexing the ferrous iron, etc. with potassium cyanide in an alkaline solution. It was also necessary to add some ammonium tartrate to the solution to prevent precipitation of ferrous hydroxide, etc. when the solution was made alkaline.

Since the impurities in the solution were never likely to exceed 1 μM, it was decided to add ten times the quantities of tartrate and cyanide, theoretically required for complexing that amount, i.e. 2 mg. of ammonium tartrate and 4 mg. of potassium cyanide for 100 ml. of solution. Titrations with these quantities of material were carried out. An approximately 0.0025 M solution of ethylene diamine tetra-acetate was standardised by titrating 10 μM

The titrations of cyanide-treated solutions of magnesium and the alkaline earth chlorides with ~ 0.0025 M di-sodium ethylene diamine tetra-acetate solution.

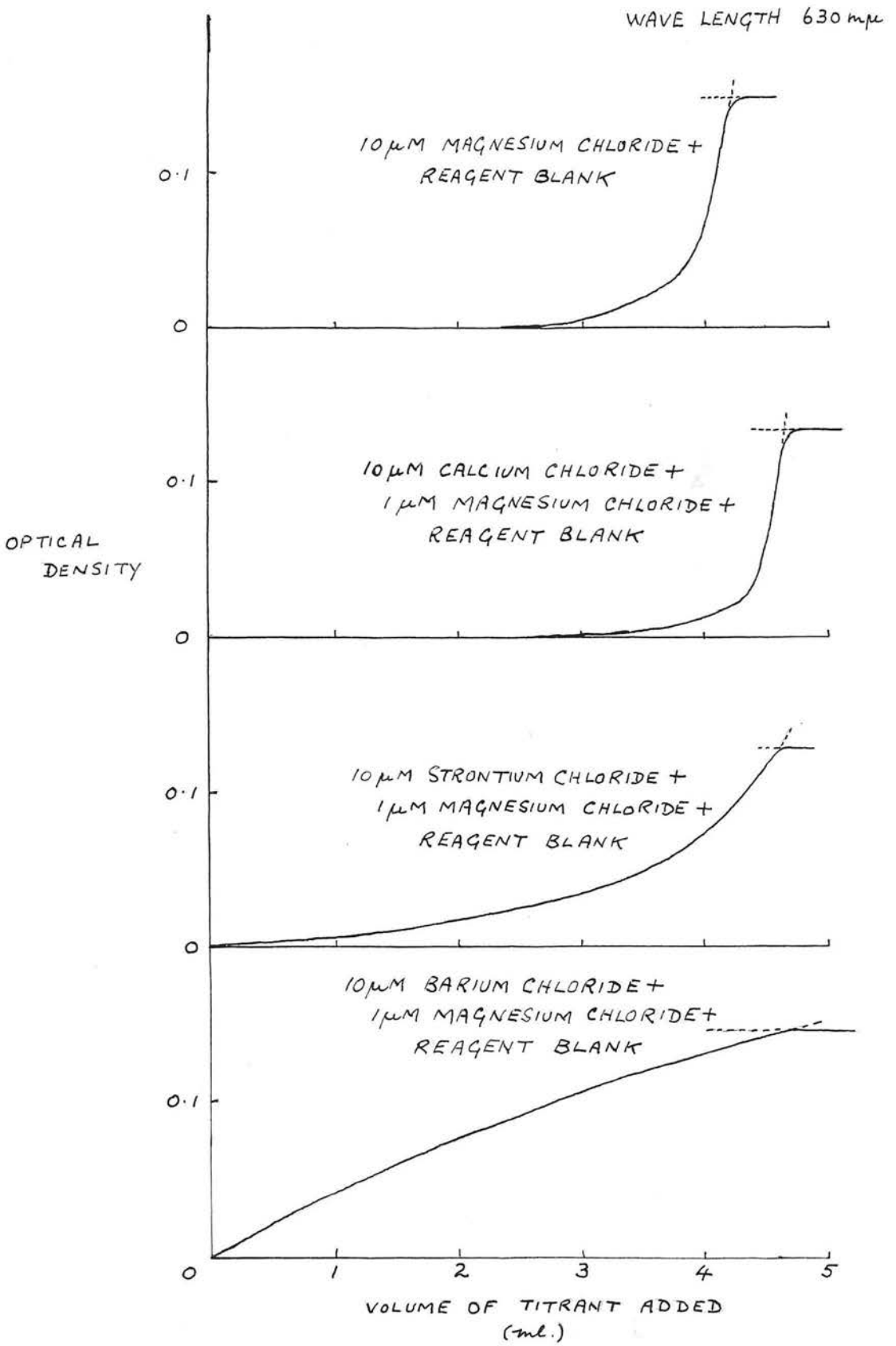


FIGURE 11.

amounts of magnesium chloride in a solution buffered at pH 10 with ammonia and ammonium chloride and containing solochrome black indicator. Impurities were complexed by treatment with cyanide. Titres for 10 μM amounts of magnesium chloride corrected for the blanks agreed to within 2 parts in 1000. With cyanide present there was no fading of the indicator. The curves of optical density against volume of titrant added, for the solutions containing cyanide are similar to those obtained with no cyanide present but the slopes are less steep. Typical curves for 10 μM amounts of magnesium, calcium, strontium and barium are shown in Figure 11. As expected, the curves for magnesium and calcium are the best. The strontium curve is less steep and the barium curve rises almost linearily from the start of the titration to the end-point. The calcium curves show no change when the ratio of magnesium : calcium increases but the curves for strontium and barium become steeper and their shapes approach nearer to the shape of the magnesium curve as the ratios of magnesium : strontium and magnesium : barium increase. For amounts of strontium less than 2.5 μM the curve resembles those of magnesium and calcium very closely. The titrations of solutions treated with cyanide were considered to be satisfactory.

Detailed procedure for the standardisation of the ethylene diamine tetra-acetate solution:

Add 10 ml. of exactly 0.001 M magnesium chloride solution i.e. 10 μM, to a conical flask and dilute to about 40 ml. with water. Add 0.05 ml. of concentrated hydrochloric acid solution, 1 ml. of 0.2% W/V ammonium tartrate solution and 0.05 ml. of a fresh 1% W/V solution of ascorbic acid to the flask. Add 2 ml. of the ammonia-ammonium chloride solution B and 1 ml. of 0.4% W/V potassium cyanide solution. Heat the solution to 80°C and allow to cool in the air for 10 minutes. Cool to room temperature by immersing the flask in cold water and add 0.1 ml. of the indicator solution i.e. enough indicator to give a rise in optical density of 0.1 to 0.2 divisions. Transfer the solution to the 'Perspex' titration cell, making the volume up to 100 ml.

Balance the Unicam SP 500 spectrophotometer with the switch in position '1' and optical density at 0. Set to an optical density of 0.01 and with the dark current shutter open add the ethylene diamine tetra-acetate solution slowly from a 5 ml. burette until the galvanometer balances. Close the dark current shutter and, after adjusting the dark current if necessary,

proceed with the addition of the titrant in small increments making sure that the optical density is steady after each addition. Near the end-point, add the solution in 0.025 ml. increments and allow an interval of 1 minute before reading the optical density. Continue the titration until the readings are the same for four consecutive additions of titrant. Obtain the end-point from a graph of optical density as a function of the volume of titrant added.

Obtain the reagent blank in a similar way.

Analysis of Synthetic Mixtures

Mixtures of 2.5 μM and 0.5 μM each of magnesium, calcium, strontium and barium were separated in duplicate, by paper chromatography. The separated cations were each extracted from the paper with acid, and the resulting solutions treated with magnesium chloride and cyanide, and titrated with ethylene diamine tetra-acetate solution. Filter paper blanks were treated in a similar way.

Detailed Procedure for the Analysis:

Prepare a 16 cm. wide sheet of Whatman No. 41 filter paper in the appropriate way (separation of the alkaline earths p. 39). Notch the bottom edge of the paper so that the solvent-mixture will drip off evenly when it reaches the foot. (The solvent-front travels faster on Whatman No.

41 paper than on Whatman No. 1, and reaches the end of the paper sheet in about 12 hr.). Mark the paper along the 7 cm. line at points 2 cm., 6 cm., 10 cm. and 14 cm. from one edge. Place an exact volume of solution containing 2.5 μM each of the four chlorides on the 2 cm. position from an 'Agla' micrometer syringe. Apply identical quantities of the chlorides on the 6 cm. and 10 cm. positions. Leave the 14 cm. position blank. Place the paper in the chromatographic vessel. After 16 - 18 hr. remove the paper and dry it in an oven. Detect the positions of the separated salts on the control (the outside strip) by spraying. Mark out the unsprayed part of the sheet into areas containing the separated cations. In a similar manner, mark out the blank strip into four areas, corresponding to the four elements (see Figure 4).

Extract the inorganic material from each of the twelve portions of paper, in funnels with polythene tube attachments (see alkali metals p.78), with 2 ml. N hydrochloric acid which should be left in contact with the paper for 10 minutes. Collect the solutions in conical flasks and wash the papers with 25 ml. of 0.02 N hydrochloric acid followed with 10 ml. of water.

To each solution add 1 ml. of 0.001 M

magnesium chloride solution and 1 ml. of 0.2% W/V ammonium tartrate solution. Add 0.05 ml. of a fresh 1% W/V solution of ascorbic acid, 2 ml. of ammonia solution C and 1 ml. of 0.4% W/V potassium cyanide solution. Heat the flasks to 80°C, cool in the air for 10 minutes, and then cool to room temperature by immersing the flasks in cold water. Add 0.1 ml. of indicator solution to each flask and transfer the solutions to the titration cell making the volume up to 100 ml. Titrate each solution with ethylene diamine tetra-acetate solution by the method outlined for the standardisation of the titrant.

Proceed with the analysis of a mixture of 0.5 μM amounts in a similar way.

Results:

The results for the analysis of mixtures of 2.5 μM and 0.5 μM of magnesium, calcium, strontium and barium are given in Table XII.

Table XII*

Cation	Initial Amount of each cation in mixture (μM)	Corrected Recovery (μM)	Error (μM)
Magnesium	2.50	2.30	-0.20
	2.50	2.50	0
	0.50	0.54	+0.04
	0.50	0.35	-0.15

Cation	Initial Amount of each cation in mixture (μM)	Corrected Recovery (μM)	Error (μM)
Calcium	2.50	2.45	-0.05
	2.50	2.70	+0.20
	0.50	0.30	-0.20
	0.50	0.48	-0.02
Strontium	2.50	2.36	-0.14
	2.50	2.52	+0.02
	0.50	0.50	0
	0.50	0 .57	+0.07
Barium	2.50	2.60	+0.10
	2.50	2.64	+0.14
	0.50	0.58	+0.08
	0.50	0.59	+0.09

* For a solution containing a particular Group IIA element, the titratible inorganic material originated from four sources, viz. (a) the Group IIA element that had been extracted from a portion of the chromatogram, (b) the foreign cations extracted from the paper at the same time, (c) the added magnesium chloride (1 μM) and (d) the titratible cations from the reagents that were added to the solution before it was titrated, i.e. the reagent blank.

For the corresponding blank solution, the titratible inorganic material originated from three sources, viz. (1) the foreign cations extracted from the paper blank i.e. the portion

of paper equal in area to the portion from which the Group IIA element was extracted, both portions being taken from the chromatogram at the same distance from the starting line, (2) the added magnesium chloride (1 μM) and (3) the titratible cations from the reagents that were added to the solution before it was titrated.

The quantities (b), (c) and (d) are the same as (1), (2) and (3) respectively and therefore the quantity of Group IIA element extracted from the chromatogram i.e. the corrected recovery, is readily obtained by subtraction.

For the titrations in this section, the foreign titratible material from the paper [(b) and (1)] varied from 0.11 μM for barium to 0.42 μM for magnesium (40 sq. cm. of paper in both cases). The reagent blank [(d) and (3)] was 0.34 μM.

The recoveries are correct to ± 0.20 μM.

Analysis of Carbonate Rocks

The above method for the analysis of synthetic mixtures was adapted for the analysis of a dolomite, a strontianite and a barytocalcite. 50 mg. of the dolomite and 150 mg. portions of the strontianite and barytocalcite were suitably treated to obtain the Group IIA cations in a 5 ml. volume of solution. 0.04 ml. portions of these solutions were applied to the chromatograms.

Detailed Procedure:

Place a weighed portion of the carbonate rock in a porcelain basin and cover with a clock glass. Moisten the powder with a little water and introduce through the spout of the basin, 2 ml. of concentrated hydrochloric acid. When the reaction has ceased rinse the cover glass and sides of the basin with water and evaporate the solution to dryness on a gently heated sand bath. Add 1 ml. of concentrated hydrochloric acid and after about 1 minute, dilute with 4 ml. water. Warm the covered basin and filter through a 7 cm. Whatman No. 4 paper receiving the filtrate in a 30 ml. beaker. Add 2 or 3 drops of dilute hydrochloric acid to the basin and rinse the insoluble residue with 8 ml. of hot water added in small portions.

Evaporate the solution in the beaker to 1 ml. and transfer the solution to a 5 ml. graduated flask. Make up to the mark with water. On a sheet of Whatman No. 41 paper 12 cm. wide, mark points along the 7 cm. line at 2 cm., 6 cm. and 10 cm. from one edge (see the chromatographic separation of the alkaline earths p.39). To each of the 2 cm. and 6 cm. marks apply 0.04 ml. of solution in volumes of 0.005 ml., drying the spots between each addition with an

TABLE XIII

Mineral	Analysis by the following method (%)	Previous Analysis (%)
* Dolomite	MgO 20.2 CaO 29.5	MgO 21.5 CaO 30.5
Strontianite	CaO 4.1 SrO 60.2	-
† Barytocalcite	CaO 19.1 BaO 42.6	CaO 18.4 BaO 44.5

* Dolomite No. 88, Bureau of Standards, Washington.

† Barytocalcite from Alston, Northumberland. (This mineral had been previously analysed for calcium oxide and barium oxide by the classical method as follows. The rock was disintegrated with hydrochloric acid, and silica and insoluble residue filtered off. The iron, aluminium, etc. were precipitated as hydroxides, which were filtered off, and the barium was removed from the solution as chromate by the method described in 'Applied Inorganic Analysis' by Hillebrand and co-authors[65]. The calcium was precipitated as carbonate and weighed as sulphate)

Electric Hair Dryer. The 10 cm. position is left blank. Develop the chromatogram in the usual manner and determine the separated cations in the way outlined in the section on the Analysis of Synthetic Mixtures (p. 99).

Results:

The results for the analysis of three carbonate rocks are given in Table XIII.

Much smaller quantities of rock could be analysed if the cations were obtained in a volume of 0.05 ml. for application to the paper. It is suggested that the solution could be evaporated to dryness in a micro-crucible, the residue taken up in 0.05 ml. of water, the resulting solution drawn up into a fine capillary and applied to the paper sheet. The crucible could be rinsed with 2 x 0.05 ml. portions of water and the washings applied to the paper as well. Approximately the same weight of rock would be similarily treated for the control. In this way, as little as 1 mg. of a dolomite and 3 mg. of a strontianite or barytocalcite could be analysed for the metal oxides, with an accuracy for each oxide of $\pm$ 1% of the total

contents, for amounts of the oxide up to 20%, and with an accuracy of ± 2% where the amount of oxide is greater than 20%.

The spectrophotometric titration procedure, that was described on page 97, has also been applied successfully in this department to the determination of microgram amounts of magnesium in copper-stabilised chlorophyll[66]. 3 mg. samples were analysed.

CONCLUSION.

For the separation of beryllium, magnesium, calcium, strontium and barium by paper chromatography, a solvent-mixture, which is claimed to be superior to any previously devised for these elements, is described. This solvent-mixture may be used for a qualitative and semi-quantitative analysis of a mixture of these cations.

A method is described, whereby micromole amounts of magnesium and the alkaline earths, separated by paper chromatography, may be titrated spectrophotometrically with di-sodium di-hydrogen ethylene diamine tetra-acetate reagent. The method should be particularly useful for the analysis of Group IIA elements in a material of which no more than 5 mg. is available. The application of the classical methods of analysis to such a sample would be very difficult if they could be applied at all.

A solvent-mixture is described for the qualitative and semi-quantitative analysis of a mixture of alkali metal and ammonium salts. Although all six cations are not separated on the one chromatogram, selective spraying reagents make such an analysis possible. Methods are also suggested for the quantitative determination

of potassium, rubidium and caesium, after their separation by paper chromatography.

A solvent-mixture for the qualitative analysis of mixtures of the Group IIIB elements and certain aluminium alloys, is proposed.

It is worth noting that the whole analysis for the detection and semi-quantitative estimation of the ten metals in Groups IA and IIA has been carried out on 0.03 ml. of solution containing never more than 20 μM of cations (i.e. < 3 mg.). 0.01 μM amounts of any of these cations, with the exception of lithium for which 0.25 μM is required, can be separated and detected in a mixture of 10 μM of the cations in the same group.

The results of certain of these researches have been published in the 'Analyst' in a paper entitled, "The Separation of Group IIA Elements of the Periodic Table by Paper Chromatography". A reprint of the paper is enclosed. Another paper entitled, "Chromatographic Separations in Phenol - Methanol - Hydrochloric Acid Solvents; with special reference to Alkali Metals" will shortly appear in the 'Analyst'.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr R. J. Magee for his guidance, encouragement and enthusiasm throughout the course of this work. I should also like to thank Dr C. C. Miller for her advice on a number of occasions, Mr T. Sheddan for taking the photographs and Mr D. H. Thow for carrying out some of the spectrophotometric titrations.

BIBLIOGRAPHY

(1) Hillebrand, W.F., Lundell, G.E.F., Bright, H.A. and Hoffmann, J.I., Applied Inorganic Analysis, Second Edition, Wiley, New York, 1953.

(2) Balkczo, H. and Schenk, W., Mikrochim. Acta 1954, 163.

(3) Consden, R., Gordon, A.H. and Martin, A.J.P., Biochem. J. 1944, 38, 224.

(4) Miller, C.C. and Magee, R.J., J. Chem. Soc. 1951, 3183.

(5) Steel, A.E., Nature 1954, 173, 315.

(6) Burstall, F.H., Davies, G.R., Linstead, R.P. and Wells, R.A., J. Chem. Soc. 1950, 516.

(7) Pollard, F.H., McOmie, J.F.W. and Elbeih, I.I.M., J. Chem. Soc. 1951, 466.

(8) Erlenmeyer, H., von Hahn, H. and Sorkin, E., Helv. Chim. Acta. 1951, 34, 1419.

(9) Seiler, H., Sorkin, E. and Erlenmeyer, H., Helv. Chim. Acta. 1952, 35, 120.

(10) Surak, J.G., Leffler, N. and Martinovich, R., J. Chem. Educ. 1953, 30, 20.

(11) Beukenkamp, J. and Rieman, W., Analyt. Chem. 1950, 22, 582.

(12) Kayas, G., J. Chim. phys. 1950, 47, 408.

(13) Buser, W., Helv. Chim. Acta. 1951, 34, 1635.

(14) Wickbold, R., Z. Analyt. Chem. 1951, 132, 401.

(15) Sweet, R.C., Rieman, W. and Beukenkamp, J., Analyt. Chem. 1952, 24, 952.

(16) Tompkins, E.R., Khym. J. X. and Cohn, W.E., J. Amer. Chem. Soc. 1947, 69, 2769.

(17) Tompkins, E.R., J. Amer. Chem. Soc. 1948, 70, 3520.

(18) Gôto, H., Sci. Reports Tôhoku Univ. 1937, 26, 391.

(19) Näsänen, R., Acta Chem. Scand. 1952, 6, 352.

(20) Dryssen, D., Svensk. kem. Tidskr. 1955, 67, 311.

(21) Campbell, D.N. and Kenner, C.T., Analyt. Chem. 1954, 26, 560.

(22) Lerner, M. and Rieman, W., Analyt. Chem. 1954, 26, 610.

(23) Bovy, R. and Dryckaerts, D., Analyt. Chim. Acta 1954, 11, 134.

(24) Nelson, F. and Kraus, K.A., J. Amer. Chem. Soc. 1955, 77, 801.

(25) Johnston, W.D. and Freiser, H., J. Amer. Chem. Soc. 1952, 74, 5239.

(26) Miller, H.S. and Kline, G.E., J. Amer. Chem. Soc. 1951, 73, 2741.

(27) West, P.W., Lyons, T.G. and Carlton, J.K., Analyt. Chim. Acta. 1952, 6, 400.

(28) Sundaram, A.K. and Banerjee, S. Analyt. Chim. Acta. 1953, 8, 526.

(29) Fouarge, J., Analyt. Chim. Acta. 1955, 12, 231.

(30) Barnabas, T., Badve, M.G. and Barnabas, J., Analyt. Chim. Acta. 1955, 12, 542.

(31) Tristram, D.R. and Phillips, C.S.G., J. Chem. Soc. 1955, 580.

(32) Gordon, H.T. and Hewel, C.A., Analyt. Chem. 1955, 27, 1471.

(33) Burma, D.P., Analyt. Chim. Acta. 1953, 9, 513.

(34) Sakaguchi, T. and Yasuda, H. J. Pharm. Soc. Japan 1951, 71, 1469.

(35) Barber, H.H. and Kolthoff, I.M. J. Amer. Chem. Soc. 1928, 50, 1625.

(36) Sergienko, P.S., Ukrain, khim. Zhur. 1932, 7, 36.

(37) Miller, C.C. and Traves, F., J. Chem. Soc. 1936, 1390.

(38) Hillebrand, W.F., Lundell, G.E.F., Bright, H.A. and Hoffmann, J.I., Applied Inorganic Analysis, Second Edition, Wiley, New York, 1953, p. 925.

(39) Arden, T.V., Burstall, F.H., Davies, G.R., Lewis, J.A. and Linstead, R.P., Nature 1948, 162, 691.

(40) Lederer, M., Analyt. Chim. Acta. 1951, 5, 185.

(41) Barnes, R.B., Richardson, D., Berry, J.W. and Hood, R.L., Analyt. Chem. 1945, 17, 605.

(42) Berry, J.W., Chappell, D.G. and Barnes, R.B., Analyt. Chem. 1946, 18, 19.

(43) Parks, T.D., Johnson, H.O. and Lykken, L., Analyt. Chem. 1948, 20, 822.

(44) Cuttica, V., Gazzetta 1923, 53, 185.

(45) Haywood, F.W. and Wood, A.A.R., J. Soc. Chem. Ind. 1943, 42, 37.

(46) Duval, C. and Doan, M., Mikrochim. Acta 1953, 200.

(47) Flaschka, H., Holasek, A. and Amin, A.M., Z. Analyt. Chem. 1953, 138, 161.

(48) Flaschka, H., Amin, A.M. and Holasek, A., Z. Analyt. Chem. 1953, 138, 241.

(49) Gillam, W.S., Ind. Eng. Chem., Anal. Ed., 1941, 13, 499.

(50) Lindner, R. and Kirk, P.L., Mikrochemie 1937, 22, 291.

(51) Schwarzenbach, G. and Ackermann, H., Helv. Chim. Acta. 1947, 30, 1798.

(52) Biedermann, W. and Schwarzenbach, G., Chimia (Switz.) 1948, 2, 56.

(53) Schwarzenbach, G., Biedermann, W. and Bangerter, F., Helv. Chim. Acta. 1946, 29, 811.

(54) Schwarzenbach, G. and Gysling, H., Helv. Chim. Acta. 1949, 32, 1314.

(55) Munger, J.R., Nippler, R.W. and Ingols, R.S., Analyt. Chem. 1950, 22, 1455.

(56) Manns, T.J., Reschovsky, M.U. and Certa, A.J., Analyt. Chem. 1952, 24, 908.

(57) Sijderius, R., Analyt. Chim. Acta. 1954, 10, 517.

(58) Sweetser, P.B. and Bricker, C.E., Analyt. Chem. 1953, 25, 253.

(59) Karsten, P., Kies, H.L., von Engelen, H. Th. J. and de Hoog, P. Analyt. Chim. Acta. 1955, 12, 64.

(60) Fortuin, J.M.H., Karsten, P. and Kies, H.L., Analyt. Chim. Acta. 1954, 10, 356.

(61) Chalmers, R.A., Analyst, 1954, 79, 519.

(62) Hunter, J.A. and Miller, C.C., Analyst, 1956, 81, 79.

(63) Heald, I.A., Coates, K.B. and Edwards, J.E., Ind. Chemist 1950, 26, 428.

(64) Sweetser, P.B. and Bricker, C.E., Analyt. Chem. 1954, 26, 195.

(65) Hillebrand, W.F., Lundell, G.E.F., Bright, H.A. and Hoffmann, J.I., Applied Inorganic Analysis, Second Edition, Wiley, New York, 1953, p. 618.

(66) Sunners, B. Unpublished work.

The Separation of Group IIA Elements of the Periodic Table by Paper Chromatography

BY

ROBERT J. MAGEE and JAMES B. HEADRIDGE

Reprinted from THE ANALYST, *the Journal of the Society for Analytical Chemistry, November*, 1955, *Vol.* 80, *No.* 956, *pp.* 785–788

The Separation of Group IIA Elements of the Periodic Table by Paper Chromatography

By ROBERT J. MAGEE and JAMES B. HEADRIDGE

(*Presented at the meeting of the Scottish Section on Thursday, April 28th, 1955*)

A mixture of methanol - *n*-butanol - *symm.*-collidine - 6 *N* acetic acid (40 : 20 : 20 : 20 per cent. v/v) has been used to separate beryllium, magnesium, calcium, strontium and barium chlorides. Sodium rhodizonate has been employed for the detection and estimation of 0·1 to 10 μM (micromoles) of strontium and barium, and 8-hydroxyquinoline has been used for the same amounts of beryllium, magnesium and calcium. Up to 10 μM of any of the group IIA metals, singly or combined, did not interfere with the detection and estimation of 0·1 μM of any particular metal, except calcium, of which the minimum amount detectable, in the presence of 10 μM of magnesium, was 0·25 μM. The presence of the alkali or the heavy metals caused no interference with the separation.

Despite the wide application of paper chromatography to inorganic analysis, few successful separations of the group IIA metals beryllium, magnesium, calcium, strontium, barium and radium have been reported.[1,2,3,4,5,6,7] Usually little or no reference is made to variation of the amounts of material separated or the minimum amounts detectable in the presence of variable amounts of other cations. Miller and Magee[4] separated strontium from barium, calcium and magnesium, and could detect and estimate 2·5-μg amounts of strontium in a maximum of 500 μg of any one or total of the other cations; no claim, however, was made for the separation and estimation of all four cations.

In this paper the separation of beryllium, magnesium, calcium, strontium and barium in a mixture and the estimation of a particular cation in a 100-fold excess of any other or total of the others are described.

Experimental

To investigate the influence of an organic anion on the solubility and distribution of the salts of group IIA metals in organic solvents, *n*-butyrates were first prepared. An ion-exchange resin, Amberlite IRA-400 (mesh 50 to 100), was used in the preparation. Group IIA butyrates were found to be soluble in water, anhydrous butyric acid and methanol; slightly soluble in higher alcohols, the solubility decreasing as the carbon chain lengthens; and insoluble in ethyl butyrate, ether, acetone, carbon tetrachloride and benzene.

Single alcohols and mixtures of alcohols were first tried in the paper-chromatographic work and later these were mixed with butyric acid of various concentrations. Table I shows the results obtained with a selection of the solvents employed.

Table I

Effect of some solvent mixtures on the alkaline-earth butyrates

	Solvent mixture	Amount of each ion, μg	R_F values			
			Magnesium	Calcium	Strontium	Barium
1.	Methanol - butanol (50 : 50 % v/v)	200	0·66 to 0·84	0·55 to 0·73	0·0 to 0·36	0·0 to 0·17
2.	Ethanol	200	0·65 to 0·90	0·15 to 0·72	0·0 to 0·37	0·0 to 0·13
3.	Methanol - butanol - 2 *N* butyric acid (40 : 40 : 20 % v/v)	200	0·69 to 0·82	0·62 to 0·75	0·24 to 0·56	0·03 to 0·43
4.	Ethanol - 6 *N* butyric acid (80 : 20 % v/v)	50	—	0·54 to 0·72	0·16 to 0·47	0·03 to 0·33
5.	Methanol - butanol - 6 *N* butyric acid (40 : 40 : 20 % v/v)	200	0·67 to 0·77	0·65 to 0·76	0·24 to 0·58	0·01 to 0·37

Solvent mixture No. 5 gave the best results, separating barium, strontium and calcium in amounts up to 50 μg, but not magnesium and calcium. The R_F values are measured from the front and back of the bands. The minimum amount of calcium, strontium and barium detectable was 10 μg.

The addition of the base *symm.*-collidine (2:4:6-trimethylpyridine) to solvent mixture No. 5 gave a marked improvement in the separations and the banding. The solvent mixture used was methanol - *n*-butanol - 6 *N* butyric acid - *symm.*-collidine (40:20:20:20 per cent. v/v). A mixture of 100-μg amounts of calcium, strontium and barium was well separated, but 50 μg of magnesium and calcium still touched. The R_F values for a mixture of 50 μg of each of the cations were: barium, 0·11 to 0·26; strontium, 0·30 to 0·47; calcium, 0·64 to 0·71; and magnesium, 0·71 to 0·81. Beryllium butyrate could not be prepared by the ion-exchange method, because, on concentrating the solution obtained, hydrolysis occurred and beryllium hydroxide was precipitated.

Further investigations with the nitrates, chlorides and acetates of the metals showed that they gave similar R_F values to the butyrates and were clearly more advantageous to use. With the discovery that other salts could be used, butyric acid in the solvent mixture was replaced by acetic acid, and a mixture of the following composition was used: methanol - *n*-butanol - *symm.*-collidine - 6 *N* acetic acid (40:20:20:20 per cent. v/v). This solvent mixture was adopted for all further work with the metals in the chloride form. All five cations were separated.

Method

Apparatus and reagents—

Descending chromatography was employed. Chromatograms were produced in a Shandon 12-inch Universal-Strip Glass Chromatank, having an over-all height of 22½ inches and a solvent trough 10¾ inches long. This apparatus accommodates sheets of chromatographic paper 16 cm wide and permits the solvent-front to travel a distance of 50 cm. No special arrangements were made for maintaining constancy of temperature, which was usually between 12° and 15° C. All experiments, except the preliminary investigations, were made on strips of Whatman No. 3MM filter-paper.

In each test a 0·01-ml portion of the solution was applied as a spot to the paper from capillary tubes calibrated by means of an Agla micrometer syringe. Four such spots could be accommodated by placing them 4 cm apart, across the paper.

Molar stock solutions of the cations were prepared from the purest material available, the following substances being used—

Beryllium—Beryllium oxide dissolved in hydrochloric acid.

Magnesium—Magnesium metal dissolved in hydrochloric acid.

Calcium—Calcium carbonate dissolved in hydrochloric acid.

Strontium—Strontium chloride, $SrCl_2.6H_2O$, dissolved in distilled water.

Barium—Barium chloride, $BaCl_2.2H_2O$, dissolved in distilled water.

Since five cations with a range of atomic weights from 9(Be) to 137(Ba) are being investigated, all quantities are expressed throughout in micromoles (μM) in preference to microgram (μg) amounts.

The following reagents were used in the detection of the cations on the paper strip by spraying—

Barium and strontium—A saturated aqueous solution of sodium rhodizonate.

Calcium, magnesium and beryllium—A 5 per cent. w/v solution of 8-hydroxyquinoline in methanol - chloroform - water mixture (85:10:5 per cent. v/v).

Procedure—

Place the solvent mixture methanol - *n*-butanol - *symm.*-collidine - 6 *N* acetic acid (40:20:20:20 per cent. v/v) in the solvent trough at least 1 hour before the experiment is started. On a Whatman No. 3MM filter-paper strip place 0·01 ml of the test solution and allow it to dry before inserting the strip in the chromatographic vessel. Allow the solvent to run down the paper for a distance of 45 to 50 cm; this usually requires 16 to 18 hours and it is very suitable to leave the paper overnight. Remove the paper from the apparatus and hang it in the air to dry for about 30 minutes; next heat it over a hot-plate until very little smell of the solvent mixture persists. The paper is now ready for spraying.

Detection and estimation of barium, strontium, calcium, magnesium and beryllium—

Spray the paper with the 8-hydroxyquinoline reagent adjusted in the following manner. To 5 ml of reagent add 0·1 ml of 0·1 *N* carbonate-free sodium hydroxide solution. Allow the paper to hang in the air for 15 minutes to ensure complete drying and examine it under an ultra-violet lamp. Under these conditions calcium, magnesium and beryllium fluoresce brilliantly (calcium, green; magnesium, yellow; beryllium, yellow), while barium and strontium

show no fluorescence at all. For estimation purposes compare the bands with a set of standards, prepared from 0·1, 0·25, 0·5, 1, 2·5, 5 and 10-μM amounts of the metals on Whatman No. 3MM filter-paper. The lengths and intensity of the bands increase with increasing amounts of the elements.

Spray the upper portion of the paper with the sodium rhodizonate reagent. Allow to dry completely and compare with standards as before.

NOTES—

1. The colour of the barium and strontium bands or the fluorescence of the calcium, magnesium and beryllium does not deteriorate on standing.

2. The use of 0·1 *N* sodium hydroxide solution in the 8-hydroxyquinoline reagent produces a solution of pH about 9·4, under which conditions only calcium, magnesium and beryllium fluoresce. At higher pH values lithium, if present, shows a bright fluorescence. It is essential to add the sodium hydroxide solution to the reagent immediately before spraying; otherwise, changes occur in the reagent and inferior fluorescent bands result. Sodium hydroxide, in preference to ammonium hydroxide, produces a more stable band.

3. The presence of 8-hydroxyquinoline on the paper does not interfere with the detection of barium and strontium.

4. *symm.*-Collidine hydrochloride forms a band below the magnesium position and can be seen as a dark spot in ultra-violet light.

RESULTS

By the above procedure 0·1 μM of barium, strontium, calcium, magnesium and beryllium can be detected. With the exception of calcium, the minimum amount of any of the metals can be detected in the presence of 10 μM of any other or total of the others. Although 0·1 μM of calcium shows the weakest fluorescence and when present with more than 5 μM of magnesium is sometimes missed, 0·25 μM of calcium can be readily detected in the presence of 10 μM of magnesium. When the amount of calcium is 5 μM or more, a precipitate is formed on the starting line. This precipitate is a calcium chloride - *symm.*-collidine complex and is only sparingly soluble in excess of the solvent mixture. This results in tailing, not only of the calcium band, but also of the others and makes the detection and estimation of minimum quantities more difficult.

The R_F values of 1-μM amounts of the five cations are: barium, 0·05 to 0·14; strontium, 0·17 to 0·27; calcium, 0·44 to 0·51; magnesium, 0·55 to 0·64; and beryllium, 0·78 to 0·93.

The positions of other elements were investigated, and it was found that most of the heavy metals were ahead of the magnesium position and caused no interference with the separation of the alkaline earths and magnesium. The R_F values of 100-μg amounts of some of these metals are shown in Table II.

TABLE II

R_F VALUES OF SOME METALS WITH THE SOLVENT MIXTURE USED

Metal	R_F	Metal	R_F
Lead	0·55 ± 0·06	Chromium	0·88 ± 0·08
Copper	0·83 ± 0·05	Manganese	0·66 ± 0·04
Mercury^{II}	0·88 ± 0·07	Zinc	0·89 ± 0·05
Iron^{III}	0·91 ± 0·07	Cobalt	0·81 ± 0·07
Aluminium ..	0·88 ± 0·10	Nickel	0·83 ± 0·07

The alkali metals sodium, potassium and lithium are separated by this procedure, the R_F values of 100-μg amounts being: sodium, 0·22 ± 0·06; potassium, 0·08 ± 0·06; and lithium, 0·52 ± 0·06.

As a final test of the validity of the proposed scheme, 14 mixtures of unknown composition were submitted to one of us (J.B.H.) for analysis. The results are shown in Table III and are the estimated amounts, in micromoles, of the metals found.

In mixtures No. 5 and No. 8, respectively, doubt was felt about the presence of the minimum amount of calcium and magnesium. Because of this doubt they are reported as missed. The results are considered to be satisfactory.

The procedure was introduced into the qualitative scheme of analysis used by third-year students in the University, during the last session. For the detection and estimation of the

elements concerned, barium, strontium, calcium and magnesium, it proved to be very successful.

TABLE III

ANALYSIS OF UNKNOWN MIXTURES; ESTIMATED AMOUNTS OF METALS

Mixture No.	Barium found, μM	Calcium found, μM	Strontium found, μM	Magnesium found, μM	Beryllium found, μM
1.	5 (4)	0·25 (0·1)	0	2 (5)	0·2
2	8	0·1	0·3 (0·1)	0·5 (1)	0
3	0	8	0·4 (0·1)	0	1
4	0	0·5 (0·2)	0	0·25 (0·1)	8 (9)
5	0	0 (0·1)	0·25 (0·1)	2·5 (9)	0·1
6	1	0·1 (0·2)	1 (0·5)	2·5 (8)	0
7	0·35 (0·2)	0·5 (0·1)	10 (9)	0	0
8	0·8 (0·5)	5 (8)	0·25 (0·1)	0 (0·1)	0·25 (0·5)
9	0·4 (0·5)	8 (10)	0 (0·1)	0	0
10	0	0·40 (0·25)	0·25 (0·1)	7 (10)	0
11	0	0·35 (0·1)	0	0·1	9 (10)
12	0·2 (0·1)	5	0·25 (0·1)	0·25 (0·1)	4 (3)
13	0·4 (0·2)	8 (7)	0·35 (0·2)	0·1	0
14	0	0·4 (0·25)	0	10	0

Note—The figures in parentheses are the amounts present when they differ from the amounts found.

The success of the separation and the positioning of the heavy metals have suggested the use of this method for the analysis of dolomites, barytocalcites, strontianites, soil samples and mineral waters. Experiments along these lines are at present being conducted and will be reported later.

We are indebted to Imperial Chemical Industries Ltd. for grants.

REFERENCES

1. Burstall, F. H., Davies, G. R., Linstead, R. P., and Wells, R. A., *J. Chem. Soc.*, 1950, 516.
2. Pollard, F. H., McOmie, J. F. W., and Elbeih, I. I. M., *Ibid.*, 1951, 466.
3. Pollard, F. H., *Brit. Med. Bull.*, 1954, **10**, 190.
4. Miller, C. C., and Magee, R. J., *J. Chem. Soc.*, 1951, 3183.
5. Erlenmeyer, H., von Hahn, H., and Sorkin, E., *Helv. Chim. Acta*, 1951, **34**, 1419.
6. Seiler, H., Sorkin, E., and Erlenmeyer, H., *Ibid.*, 1952, **35**, 120.
7. Surak, J. G., Leffler, N., and Martinovich, R., *J. Chem. Educ.*, 1953, **30**, 20.

CHEMISTRY DEPARTMENT
KING'S BUILDINGS
THE UNIVERSITY
EDINBURGH

April 12th, 1955

DISCUSSION

MR. R. B. RASHBROOK asked if the possible use of a side-strip spray method for locating positions of metals before their elution had been considered instead of the ignition of the sprayed strips.

MR. HEADRIDGE said that such a method had not yet been tried, although it was being considered. The ignition of Whatman No. 3MM paper was certainly unsatisfactory, because of the high calcium content of the ash and, whilst this could be reduced to one-tenth by the use of double-acid-washed paper, it seemed likely that the metals in the paper ash would still interfere with the titration.

MR. A. V. PARKE asked whether it was possible to make quantitative determinations of separated cations on chromatograms by photometric measurement of the colour or fluorescence of the spots.

MR. HEADRIDGE replied that, by these means, a semi-quantitative determination was possible, although other workers had found that the accuracy was generally not better than 5 per cent.

MISS E. S. R. MCCALLUM asked what method was used for the determination of calcium and magnesium after separation by paper chromatography and what time was taken for such analyses.

DR. MAGEE replied that it was intended to titrate the salts spectrophotometrically with a solution of ethylenediaminetetra-acetic acid after they had been removed from the paper. Only a few preliminary titrations had been done, and at present it was not certain how long an analysis would take.

MR. E. J. BUTLER asked whether the choice of solvent mixture was entirely empirical.

DR. MAGEE replied that this was so. While investigations of the solubilities of the salts in various solvents gave some indication of what solvents to avoid, the final solvent mixture had been reached by starting with the simplest solvents, such as alcohols, and adding other substances that improved the separation.

PRINTED BY
W. HEFFER & SONS LTD.
CAMBRIDGE, ENGLAND